PRIVY COUNCIL

MEDICAL RESEARCH COUNCIL SPECIAL REPORT SERIES
No 273

INFECTIVE HEPATITIS

STUDIES IN EAST ANGLIA DURING THE PERIOD 1943 47

BY
F. O MacCALLUM, A M McFARLAN,
J A R MILES, M R POLLOCK
and C WILSON

LONDON HIS MAJESTY S STATIONERY OFFICE
1951

Universal Decimal Classification
616 36—00?

MEDICAL RESEARCH COUNCIL

JAUNDICE COMMITTEE

PREFACE

The investigation recorded here ended in 1947 and the Report in its present orm was accepted for publication not long afterwards It was, however, ecided to postpone publication because, with the exception of the observations n epidemiology, much of the work had already been described in detail in he medical press and it was felt that precedence in the Council's Special Report Series should be given to work of which accounts were not available lsewhere. Since then some important new knowledge of hepatitis has been ained, particularly of the clinical aspects of the disease, but the findings of he Report are in general very little affected and the temptation to revise it as been deliberately resisted It remains strictly a record of a particular eries of detailed investigations and is not intended as a comprehensive treatise n the subject The Council believe that the collection of the full material nder one cover will be useful

Among the British troops in the Mediterranean area there were 4,000 cases of jaundice in 1941-42 and 12,000 in 1942-43 A large-scale outbreak occurred among American troops arriving in Northern Ireland in 1942 These events gave rise to anxiety over the possibility of even larger epidemics in the Services and of the spread of the disease to the civilian population In May 1943, therefore, at the request of the Ministry of Health and in consultation with the War Office, the Council appointed a Jaundice Committee with Professor L J Witts as Chairman, while a Jaundice Research Team, consisting of the authors of the present Report, was formed under the general direction of Professor S P Bedson, one of the members of the Committee For administrative convenience and because it was undesirable that transmission experiments should be carried out in epidemic areas, the work was concentrated in this country and the team was housed in the Department of Pathology at Cambridge University Jaundice was made notifiable in Civil Defence Region 4, which included Cambridge and had a population of about 2½ million, and sufficient clinical material was soon found

The investigations comprised epidemiological, clinical and pathological observations as well as experimental studies Transmission to animals was not achieved, despite the use of many species and of every procedure known to increase susceptibility to viruses, and it was necessary to use human volunteers The outstanding findings of the human experiments were that a virus is present in the blood in arsenotherapy jaundice and that virus is excreted in the faeces in infective hepatitis

The clinical, pathological and epidemiological studies are also of great interest, and the whole series of observations forms a notable contribution to the increased understanding of jaundice that was gained during the recent war

MEDICAL RESEARCH COUNCIL,
38 Old Queen Street,
London, S W 1

September 4th, 1951

CONTENTS

CONTENTS (*continued*)

INFECTIVE HEPATITIS

STUDIES IN EAST ANGLIA DURING THE PERIOD 1943-47

by

F O MacCALLUM, M D , B Sc , A M McFARLAN, M B ,
J A R. MILES, M B , M R POLLOCK, M B ,
and C WILSON, D M , F R C P

I EPIDEMIOLOGICAL STUDIES

by

A M McFARLAN M B

THE investigations reported here were made between May 1943 and December 1947 from the Medical Research Council s Jaundice Laboratory in the Department of Pathology of the University of Cambridge In order to present a comprehensive picture of the epidemiology of infective hepatitis it will be convenient to consider the data obtained at Cambridge under several headings and to discuss each set of observations in the light of the published findings of other workers

In May 1943 Dr A Leslie Banks then Principal Regional Medical Officer of the Ministry of Health made arrangements for the voluntary reporting of cases of catarrhal jaundice (infective hepatitis) in the Eastern Region or Region 4 which covered the counties of Norfolk East Suffolk West Suffolk, Essex Hertfordshire Cambridgeshire Isle of Ely, Bedfordshire and Huntingdonshire (Fig 1 p 3) By this means information about individual cases and epidemics was received from medical practitioners through Medical [illegible]

17th November 1943 The regulations provided for the notification by medical practitioners to the Medical Officer of Health of the district of any case of catarrhal jaundice acute inflammation of the liver acute necrosis of the liver toxic jaundice or infective jaundice The notifications or a nil return were sent each week to the Principal Regional Medical Officer at Cambridge In his office relevant data were recorded on punch cards and a summary of the week s notifications by dates of onset and places of occurrence was sent to the Jaundice Laboratory

Under the regulations the civilian cases were almost all notified as infective hepatitis (catarrhal jaundice) A few cases of arsenotherapy hepatitis (D ble and McMichael 1943) of homologous serum hepatitis (Medical Officers of the Ministry of Health 1943) and of leptospirosis (Weil s disease) were also notified under one of these names but these have been excluded from the account which follows It is possible that other cases of these diseases were notified as infective hepatitis, but investigation of cases throughout the Region gave no reason to suspect that more than a very few were not in fact infective hepatitis From time to time intimations were received that mild cases were occurring and were not notified because the patients never consulted a doctor It was not possible to investigate these suspect cases but similar mild cases were seen during the investigation of epidemics and it seems probable that they occur

sporadically also They are mentioned here because they justify the assumption that the notification of infective hepatitis as of other infectious diseases is incomplete The cases notified therefore represent a minimal incidence

Cases in the Armed Forces were also notified and investigations into their epidemiology have been reported (McFarlan, 1945a) The findings were similar to those in the civilian population except for the age incidence In home based units the incidence was mainly sporadic, but epidemics occurred in places where there was a civilian epidemic and in training units with a large turnover of recruits Epidemics occurred late in 1943 and early in 1944 in units which returned from the Mediterranean area, but notifications from the localities where units were stationed showed that those outbreaks did not give rise to epidemics in the civilian population The trend of infective hepatitis in the civilians in Region 4 was not disturbed by military epidemics and can be discussed without reference to them

Region 4

The area to which the Jaundice Regulations applied also included the County Boroughs of Great Yarmouth, Norwich, Ipswich and Southend, but parts of Essex and Hertfordshire adjoining London were excluded (see notes to Table 1)

Population The Registrar General's estimate of the population at mid 1944 was 2 6 million, of whom 1 6 million lived in urban areas and 1 0 million lived in rural districts (Table 1) Some of the urban areas are small country towns little larger than many villages Administrative "urban districts" of this kind included a population of 82,000 living in 28 towns in conditions not far removed from those in rural districts, they included also a population of 201,000 living in towns with between 5,000 and 10 000 inhabitants During the war years, 1939-45, there was from time to time a movement of population from London and certain coastal areas to reception areas in the Region However, the mid-1944 figures are a fair estimate of the population in individual districts during 1944 and 1945 The main occupation of the inhabitants is agriculture there are also light industries in some of the towns

TABLE 1

Population of Region 4 by Counties Registrar General's mid 1944 Estimate

County	Population in thousands Urban	Rural	Total
Bedfordshire	199	85	284
Cambridgeshire	78	75	153
Essex*	544	199	743
Hertfordshire*	307	137	444
Huntingdonshire	28	34	62
Isle of Ely	49	33	82
Norfolk	195	265	460
E Suffolk	156	107	263
W Suffolk	41	67	108
Total	1,597	1 002	2 599

* Excluding the Municipal Boroughs of Barking Chingford Dagenham Ilford Leyton Walthamstow and Wanstead and Woodford and the Urban Districts of Chigwell and Waltham Holy Cross

[illegible] and East Barnet and the

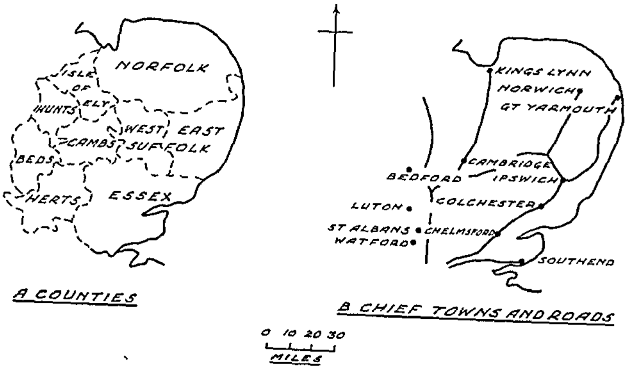


FIG 1 *Map of Region 4*

Traffic routes Three main lines of communication radiate from London, one through Essex to East Suffolk and East Norfolk, one through Hertfordshire and Bedfordshire to Huntingdonshire and beyond, and one through Hertfordshire and Cambridgeshire to the Isle of Ely and West Norfolk (see Fig 1) Cross country train and bus services are few and infrequent, and in rural districts the only journeys frequently made by adults are to shops and markets in the nearest town and by older children to the nearest Village College or Area School

Geography The whole Region is low lying The East Anglian Heights, which extend from the south-west corner towards the north east nowhere rise higher than 600 feet They are the watershed between the Ouse and its tributaries, which flow north through the Fens to the Wash, and several rivers which flow east or south east to the North Sea The greater part of the Region is agricultural land on which there were many aerodromes during the war years

Climate The climate is drier and has greater extremes of temperature than that of the west coast of England, showing a transition from the oceanic towards the continental type Figures of rainfall and of maximum and minimum temperatures were supplied by the Director of the Botanic Garden Cambridge University In 1944 and 1945 the rainfall was below the thirty year mean owing to a deficiency in the first half of each year The monthly maximum and minimum temperatures rose in June, July and August and fell in the last three months The fall from the summer level in September coincided with the rise of notifications of infective hepatitis and some respiratory infections from their summer minima (see p 12)

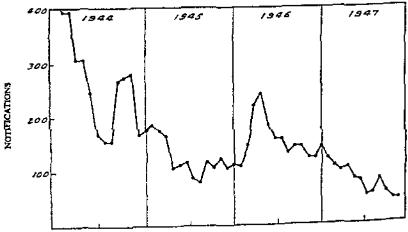


FIG 2 *Four weekly Notifications (1944-47) in Region 4*

Notifications from 1943 to 1947

SECULAR TREND

The incidence of infective hepatitis in Region 4 showed an overall downward trend from 1943 to the end of 1945 (Table 2 and Fig 2) with 2,227 notifications in 38 weeks of 1943, 3,559 in 1944 and 1,626 in 1945 In 1946 there were 1,980 notified cases, the rise being due to an increase in the south of Essex In 1947 the number of notifications was 1,034, the lowest total in the four complete years during which notification was compulsory The attack rates per 1000 in these four years were 1 3, 0 6, 0 7 and 0 4 The year 1946 therefore appears as a slight irregularity in the downward trend The figures for May to November 1943 were derived from voluntary reporting and are less complete than those for later years so that the downward trend was actually more marked than the figures indicate

Many medical practitioners in the Region stated that they had seen more cases of infective hepatitis in the winter of 1942-43 than in previous or subsequent years Epidemics outside the Region were reported from Wolverhampton in 1942 (Edwards, 1943), Leicestershire in 1942-43 (Lisney, 1944), and Wembley in 1943 (Ford, 1943) In 1941, 1942 and 1943 several village outbreaks were seen in Oxfordshire, and in 1941-42 there was an extensive outbreak in and around Preston, Lancashire (unpublished data) At the same time there was an outbreak in Axminster, Devon (personal communication from Dr W L Hawley) In 1939 an epidemic occurred in the Lavant valley, Sussex (Newman, 1942), and in 1938 there was one in Wensleydale, Yorkshire (Pickles, 1939) These records and observations suggest that infective hepatitis was prevalent in England for some years before the 1942-43 winter and that that was the time of highest prevalence in some areas Support for this conclusion is provided by the graph of the incidence of infective hepatitis in Home Commands of the Royal Air Force (Fig 3) The graph shows a rise in the 1941-42 winter, a larger rise in 1942-43, and a progressive decline in subsequent years

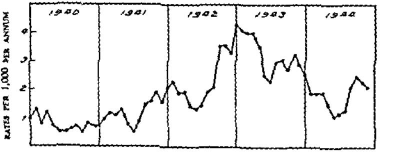


FIG 3 *Infective Hepatitis in R A F Home Command Monthly Rates per 1 000 per annum* (From a graph supplied by Air Commodore T McClurkin)

TABLE 2

Number of Civilian Cases of Infective Hepatitis Notified in Region 4 from 1943 to 1947 (by weeks and four weeks)

Four week period ending about	1943	*Total*	1944	*Total*	1945	*Total*	1946	*Total*	1947	*Total*
Jan 29 (*1*)			107 131 100 111	449	34 41 35 65	175	25 30 29 27	111	39 39 43 27	148
Feb 26 (*2*)			99 90 92 110	391	41 47 55 44	187	27 22 29 29	107	30 33 31 24	118
Mar 25 (*3*)			108 102 101 86	397	56 43 39 34	172	25 24 42 57	148	32 16 30 28	106
Apr 22 (*4*)			73 84 64 84	305	40 56 34 33	163	53 49 64 52	218	18 17 24 34	93
May 20 (*5*)	60	60	83 86 76 69	314	23 29 27 26	105	72 57 53 58	240	29 26 21 25	101
June 17 (*6*)	73 127 132 93	425	70 50 66 58	244	30 18 28 34	110	54 43 41 46	184	20 28 15 14	77
July 15 (*7*)	107 91 63 59	320	42 42 38 46	168	29 37 21 26	113	36 39 40 44	159	20 20 18 17	75
Aug 12 (*8*)	57 25 12 16	110	45 43 35 34	157	24 28 15 16	83	54 34 42 26	156	19 9 10 11	49
Sept. 9 (*9*)	23 23 42 63	151	39 33 46 34	152	14 19 19 24	76	28 30 32 40	130	15 10 14 13	52
Oct 7 (*10*)	58 44 41 56	199	51 72 80 62	265	25 29 32 28	114	31 49 29 35	144	10 22 16 33	81
Nov 4 (*11*)	75 77 65 63	280	69 63 75 67	274	27 30 25 22	104	43 37 25 37	142	8 12 19 15	54
Dec 2 (*12*)	51 49 75* 90	265	69 56 92 60	277	25 37 25 35	122	32 35 24 29	120	7 12 8 14	41
Dec 30 (*13*)	112 97 94 114	417	53 41 47 25	166	24 23 39 16	102	44 39 19 19	121	14 12 4 9	39
Total	(33 weeks)	2,227		3,559		1,626		1,980		1,034

* Reporting of cases was voluntary till this week when compulsory notification was introduced in some districts The other districts introduced compulsory notification in the following weeks of the year

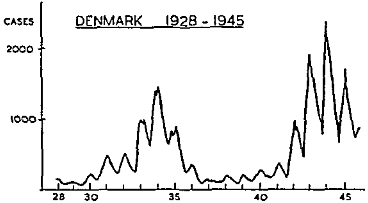


FIG 4 *Infective Hepatitis in Denmark* (1928 1945)
(From Bjørneboe, 1946)

Long term fluctuations in incidence are evident in the graph of notifications in Denmark from 1928 to 1945 (Fig 4) The numbers of epidemics recorded in the literature since 1850 (Fig 5) suggest that similar fluctuations have occurred in several countries in both eastern and western hemispheres and that from time to time the disease has been pandemic In 1942 epidemics occurred in British troops in Palestine (Cameron, 1943), in Egypt (Spooner, 1944), in West Africa (Findlay, Martin and Mitchell, 1944) and in Syria (Cullinan, unpublished report) At the same time there was a high incidence in German troops in Libya, Poland and Norway as well as in civilians in Denmark (Fig 4) and in England In May 1943 epidemics were reported in troops in Western Australia (Oxer, personal communication) No information has been received of epidemics in America in 1942 43, but in these years infective hepatitis was epidemic in many countries

The secular trend of infective hepatitis is characterized by long term variations in prevalence with epidemics or pandemics occurring at irregular intervals preceded by a slow rise and followed by a slow fall In England in 1942 and 1943 there was a widespread epidemic and the following four years in Region 4 were a period of decline in prevalence

SEASONAL INCIDENCE

The cases of infective hepatitis reported in Region 4 from May to December 1943 were arranged according to the week of onset, many cases notified in the early months were discarded on account of the lack of this information For this reason and because the voluntary reports did not cover the whole Region the figures for this period are too low, but considering the observations of later years the downward trend in July (Table 2) probably reflects the actual trend The low level during August was partly due to the absence of reports from schools though it seems that there was a true decrease in incidence because notifications from the Services fell to a low level at the same time It is probable also that there was a true rise in September and October in addition to the effect of the resumption of reports from schools The rise in late November and in December was the result of the gradual introduction of compulsory notification in the Region

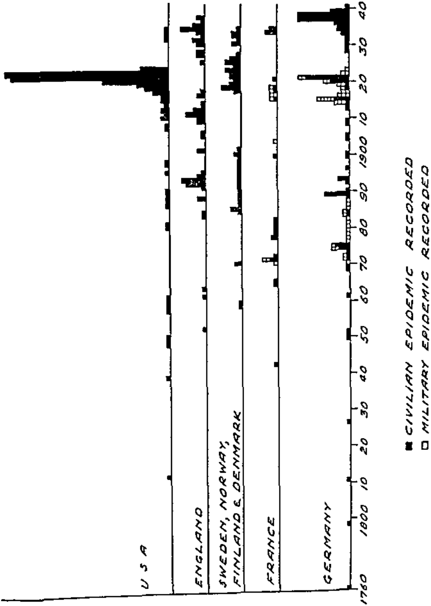


FIG 5 *Civilian and Military Epidemics of Infective Hepatitis*
(From von Bormann, Bader, Deines and Uaholtz, 1943)

Notifications in 1944 and 1945 showed a fall during the first seven months and a rise beginning in September superimposed on the general downward trend (Table 2 and Fig 2) In 1946 the seasonal trend was different The number of notifications rose in March, remained high in April and May and then fell gradually to the end of the year with a slight rise during September and October The rise in spring was most marked in Essex, the percentage of cases in each quarter of 1946 being 21 3, 41 0, 23 5 and 14 2 In the rest of the Region the percentages were 21 0, 24 0 25 0 and 30 0 and the rise in autumn was more evident Thus the unusual seasonal incidence in 1946 was due to an out-of season increase in prevalence in one part of the Region In 1947 the numbers fell throughout the year with a slight autumnal rise, repeating at a lower level of incidence the seasonal pattern observed in 1943, 1944 and 1945

A similar seasonal trend with a minimum in summer and a rise in autumn is apparent also in the graph of incidence in the Home Commands of the R A F (Fig 3) and in the monthly incidence for the years 1928 45 in Denmark (Fig 4) In Fig 6 the average monthly incidence of infective hepatitis in Sweden for the seven years 1931 37 is compared with that of some other infectious diseases

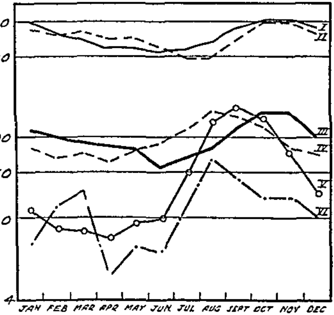


FIG 6 *Mean Monthly Value of Recorded Cases of Infective Hepatitis and some other Infectious Diseases in Sweden for the period* 1931 37
(From Selander 1939)

I Diphtheria
II Scarlet fever
III Infective hepatitis
IV Typhoid
V Infantile paralysis
VI Dysentery

TABLE 3

Notifications of Infectious Diseases in Region 4 by Four week Periods

Four week period ending	Measles	Pertussis	Pneumonia	Diphtheria	Scarlet fever	Infective hepatitis	Dysentery	Typhoid and paratyphoid fevers
1944								
Jan 29	369	603	357	78	479	449	29	1
Feb 26	731	661	256	62	478	391	64	—
Mar 25	1 152	773	332	68	556	397	84	1
Apr 22	932	701	300	57	427	305	274	—
May 20	702	877	177	43	373	314	55	3
June 17	587	971	170	34	396	244	79	—
July 15	560	1 125	99	30	343	168	29	12
Aug 12	501	811	75	30	351	157	62	12
Sept 9	191	642	62	28	265	152	254	9
Oct 7	255	304	80	42	380	265	212	6
Nov 4	405	264	143	47	437	274	210	1
Dec 2	518	390	129	51	459	277	162	2
Dec 30	673	308	144	51	334	166	71	2
Total	*7 576*	*8 430*	*2 324*	*621*	*5 278*	*3 559*	*1 585*	*49*
1945								
Jan 27	1 223	399	324	44	266	175	125	—
Feb 24	2 631	393	348	45	254	187	213	2
Mar 24	3 729	358	232	54	247	172	154	2
Apr 21	4 399	343	184	47	245	163	66	1
May 19	4 397	283	128	43	229	105	143	3
June 16	3 732	267	89	26	253	110	125	3
July 14	2 660	232	66	35	218	113	52	2
Aug 11	1 568	234	45	34	214	83	110	8
Sept. 8	623	317	45	48	167	76	172	3
Oct 6	166	194	62	55	266	114	156	5
Nov 3	256	187	79	58	292	104	53	4
Dec 1	484	339	95	88	327	122	62	4
Dec 29	938	286	177	117	289	102	137	2
Total	*26 806*	*3 832*	*1 874*	*694*	*3 267*	*1 6[illegible]8*	*1 568*	*39*

In British troops in the Mediterranean area in 1917-18 (Willcox, 1919) and again in 1942, 1943 and 1944 epidemics began in early autumn and reached a peak in November, December or January In troops in Western Australia epidemics began in the autumn (May) of 1943 (Oxer, personal communication) Willard (1944) recorded a high incidence of cases in the autumn and winter months in the U S Navy in the South Pacific Thus the incidence of infective hepatitis is minimal in summer rises in autumn and is maximal in winter

Seasonal variations in incidence of other notifiable diseases in Region 4 The weekly notifications of infectious diseases in Region 4 shown in the weekly bulletin prepared by the Principal Regional Medical Officer were

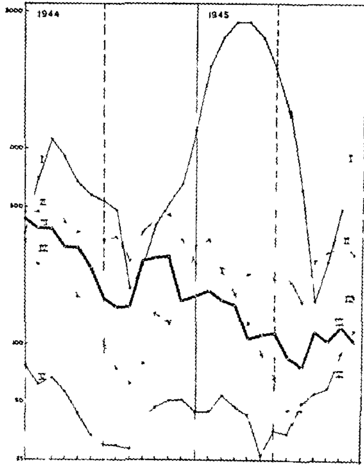


FIG 7 *Incidence of Infectious Diseases in Region 4 during 1944 and 1945*
I Measles
II Scarlet Fever
III Infective Hepatitis
IV Pneumonia
V Diphtheria

used to arrive at the four weekly totals in 1944 and 1945 (Table 3) In Fig 7 the numbers of notifications of some infectious diseases in Region 4 are plotted on a semilogarithmic scale so that the extent of their seasonal variation is apparent Measles and pneumonia showed the greatest variation In diphtheria and scarlet fever the variation was less, and the curves for these diseases are very similar to that for infective hepatitis In 1944 infective hepatitis and these four diseases had summer minima at the same time, and their rises in autumn began in the same four week period ending 7th October In 1945 the autumnal rise of infective hepatitis, scarlet fever and pneumonia began in the period ending 6th October, that of measles in the following period ending 3rd November, and that of diphtheria in the period ending 14th July The later rise of measles followed a greater prevalence of the disease in the earlier part of the year, and the earlier rise in diphtheria preceded a winter rise greater than in the preceding year Despite these discrepancies it appears that the summer minimum and autumn rise of infective hepatitis in 1944 and 1945 synchronized with the minima and autumn rises of four diseases in which infection is spread from the respiratory tract Pertussis showed no seasonal trend in these years

The notifications of dysentery fluctuated considerably owing to explosive outbreaks due to *Shig sonnei* which masked any seasonal trend The notifications of enteric fevers showed a rise in the periods ending 15th July 1944 and 11th August 1945, thus preceding the rise in infective hepatitis in both years

The experience recorded here is too short to justify any general conclusions but other observations have shown similar relationships Willcox (1919) noted in the Mediterranean area that the curve of intestinal infections preceded that of infective hepatitis, and upper respiratory infections have been prevalent at the time of several epidemics of infective hepatitis Thus it may be said that an increased number of cases of infective hepatitis not infrequently follows the increased prevalence of intestinal infections and synchronizes [illegible] from the respiratory tract

[illegible] collected by the Principal [illegible] *did not include data for* Essex In the rest of the Region in both these years the seasonal incidence of the various notifiable infections was similar to that in 1944 and 1945 The Registrar General s weekly notifications for Essex showed that in 1946 the notifications of measles rose from the beginning of the year to a peak in the first week of July Thus in Essex measles was prevalent during the [illegible] the incidence of [illegible]

is transmitted from the respiratory tract

AGE AND SEX INCIDENCE

The age and sex distributions of cases of infective hepatitis notified in 1944 and 1945 were similar in the two years (Table 4) The excess of females in these years was due almost entirely to the difference in the 20 to 40 year age group, which can be attributed to the absence of adult males on active service In 1946 and 1947 when demobilization was proceeding the cases were evenly divided between the sexes and apart from the increase of the proportion in males aged 20 to 40 years the age and sex distribution was similar to that in 1944 and 1945

TABLE 4

Percentage of Cases in Age and Sex Groups

Year	Sex	Age group (years) 0–4	5–9	10–14	15–19	20–39	40–59	60+	N.S.*	Total	Number
1944	M	1.7	10.7	12.1	7.5	7.7	3.6	1.2	0.9	45.4	1624
	F	1.7	11.5	11.4	7.8	15.3	4.1	1.6	1.2	54.6	1949
1945	M	2.2	9.8	11.1	6.3	9.9	2.9	1.7	1.1	45.0	708
	F	2.3	11.1	10.4	7.5	16.4	3.6	2.0	1.7	55.0	861
1946	M	1.9	8.9	8.7	6.6	17.8	4.2	1.5	1.1	50.7	1004
	F	2.2	8.6	7.2	7.2	18.2	3.3	1.1	1.5	49.3	976
1947	M	2.4	9.0	4.9	4.2	22.1	5.0	3.2	0.9	51.7	534
	F	2.3	7.2	8.1	6.9	16.4	3.9	2.7	0.8	48.3	500

* Age not stated

Population figures, by age and sex were not available for the calculation of attack rates but it can be concluded that the incidence was highest in children of school age The percentage of cases in the age group 5 to 14 years was 45.7, 42.4 33.4 and 29.2 in successive years all of these figures are considerably higher than expected in a group forming about 15 per cent of the population The incidence in children under 5 years of age was low and there was a fall in the incidence with increase of age above 15 years

The low incidence in children under 5 years of age is due at least in part to the more frequent occurrence of non icteric or symptomless infections in them (p 72) In children of school age the incidence is equally high in the 5 to 9 and 10 to 14 age groups In three places where epidemics in day schools were investigated (Table 5) it was possible with the help of Medical Officers of Health and schoolteachers to collect data for the calculation of age and sex attack rates In all three places the attack rate in the 10 to 14 age group was higher than in the 5 to 9 age group This age

TABLE 5

Epidemic Attack Rates in Children Attending Day schools

Age in years	5—9			10—14			5—14		
Sex	Male	Female	Total	Male	Female	Total	Male	Female	Total
			Numbers of Cases						
Southend (1943)	72	82	154	120	84	204	*192*	*166*	*358*
Bishop's Stortford (1943-44)	16	28	44	31	24	55	*47*	*52*	*99*
Bungay (1943)	18	19	37	66	54	120	*84*	*73*	*157*
			Numbers at Risk						
Southend	2 054	1 964	4 048	2 216	1 960	4 176	*4 300*	*3 924*	*8 224*
Bishop's Stortford	317	274	591	255	186	441	*572*	*460*	*1 032*
Bungay	142	126	268	331	159	512	*495*	*285*	*780*
			Attack Rates (per cent.)						
Southend	3.5	4.2	3.8	5.4	4.3	4.9	*4.5*	*4.2*	*4.4*
Bishop's Stortford	5.3	10.2	7.5	12.2	12.9	12.5	*8.2*	*11.3*	*9.6*
Bungay	12.7	15.1	13.8	18.7	33.9	23.4	*17.0*	*25.6*	*20.1*

incidence differs from that of most of the common infectious diseases, where the attack rate is highest in the first 5 years of life and higher between 5 and 9 than between 10 and 14 years of age, but mumps (Collins, 1924) and rubella (Smith, 1935) have a distribution with more cases in older children and not unlike that in infective hepatitis In recent years diphtheria (Russell, 1943) and poliomyelitis (Burnet, 1940) have in some countries attacked a higher proportion of older children and young adults than formerly The change in diphtheria is probably related to the smaller size of families and to a decrease in overcrowding (Russell, 1943) The age incidence of infective hepatitis may also have changed but there are no records which suggest that it has been as common in young children as most endemic infections There are however many records which show that young adults are frequently attacked

In Region 4 in 1944, 48 8 per cent. of cases of infective hepatitis occurred in persons aged 15 years or more, and 50 3 59 9 and 65 4 per cent in the following three years These proportions are considerably higher than those found in other infectious diseases For example, in England and Wales in 1944 the Registrar General's corrected notifications give the following percentages of all cases found in persons aged 15 or more years diphtheria, 28 8, poliomyelitis, 14 4, scarlet fever, 11 2, measles, 3 8, and whooping cough, 2 1

In [illegible]

breaks [illegible]

breaks the main incidence was among children In Region 4 in 1943-45 the reverse was true, for it was only in a few relatively small outbreaks that a high proportion of the cases were in adults *For example, there were 18* adults in 38 cases in Fen Ditton in 1943, 9 adults in 10 cases in Debenham in May and June 1944, and all 8 cases in Swavesey in 1944 were in adults Epidemics in nurseries (p 33) provided other instances of a high incidence in adults The outstanding examples of outbreaks among adults are the epidemics in armies at war, from the time of the American Civil War until the present day The explanation of the military outbreaks may lie in the lack of *immunity in adults* born and bred in country districts, who are then brought into contact with others brought up in towns and both are exposed to the privations and hardships of campaigns It remains true, however, that infective hepatiti[illegible]

occur at any age H[illegible]

age incidence with a [illegible]

Oliphant, 1944a)

Age distributions of cases of infective hepatitis similar to those found in Region 4 have been reported by many authors including Williams (1923) in New York, Selander (1939) in Sweden and von Bormann *et al* (1943) in Germany Infective hepatitis therefore has rather consistently shown its highest incidence in children of school age and a relatively high incidence in young adults

URBAN AND RURAL INCIDENCE

In 1944 the attack rates per *1,000 of the mid-1944* population were for the whole Region 1 3, for urban areas 1 2, and for rural districts 1 7 The ratio of urban to rural rates was thus 0 7 to 1 in the Region (Table 6)

During epidemics the attack rate in schoolchildren was lower in the urban areas of Southend and Bishop's Stortford than in rural areas in East Suffolk (Table 7) The lower attack rates in towns were probably the result of an immunity resulting from previous exposures and non icteric or sub clinical attacks It is also possible that primitive sanitation in rural areas and particularly in the schools played a part in producing the high incidence there

TABLE 6

Attack Rates per 1,000 per Annum by Counties and by Aggregate of Urban and Rural Districts

County	*1944*	*1945*	*1946*	*1947*
Bedfordshire	1·0	0 4	0 2	0 1
Cambridgeshire	1 4	0 6	0 5	0 2
Essex	1 1	0 7	1 3	0 7
Hertfordshire	1·0	0 4	0 3	0 2
Huntingdonshire	1·0	0·7	1 3	0 2
Isle of Ely	0 2	0 1	0·1	0 1
Norfolk	1 9	0 9	0·7	0 4
E Suffolk	2·0	0 8	0 5	0·2
W Suffolk	1 8	0 4	0 3	0 3
Region 4	1 3	0·6	0 7	0 4
All Urban Districts	1 2	0 6	0 8	0 5
All Rural Districts	1 7	0 6	0 5	0·3

In 1945 the attack rates in urban areas and rural districts were equal at 0 6 per 1 000 The fall from the 1944 rates was less (50 per cent) in urban areas than in rural districts (65 per cent) In 1946 and 1947 the attack rates in urban districts were higher than those in rural districts These findings suggested that infective hepatitis during the decline in incidence was conforming to the general epidemiological law (Greenwood, 1939) of retreating into the towns after a period of increased prevalence when rural districts were more severely attacked

TABLE 7

Attack Rates per 100 Schoolchildren during Epidemics

Place	*No of schools*	*No of cases*	*Attack rate (per cent)*	*Attack rate (per cent) Max*	*Min.*
Southend	25	382	4 4	7 7	0·2
Bishop's Stortford	5	100	9 7	16 1	5 8
Leiston district	8	129	14 0	37 5	5 8
Stowmarket	2	108	15 1	22 4	7 4
Samford	3	58	15 5	53 2	6 2
Bungay	4	165	20 5	31 3	10·0

In large towns epidemics were occasionally seen (p 26) but more usually there [illegible] the year (Table [illegible] wed that apart [illegible] 1943 the cases occurred sporadically, having no known contact with a previous case and no likelihood of infection by a common article of diet. Only occasionally

was there a secondary case in a family, at the usual 20- to 40-day interval Investigations made by Medical Officers of Health in Watford (Pearson, personal communication) and in Hornchurch (Ball, 1944) showed that in these towns the disease behaved like an endemic disease and cases were distributed fairly evenly throughout the year In 1944 eight other urban areas with large populations (Luton, Thurrock, Bedford, Chelmsford, Romford, Billericay Brentwood, Ipswich), showed a similar succession of cases throughout the year (Table 8) In 1945 the number of cases decreased in all eleven areas but the endemic series of cases continued in Luton, Cambridge, Thurrock and Hornchurch and to a lesser extent in the other seven areas In Hornchurch

TABLE 8

Cases of Infective Hepatitis in some Urban Areas (by Four-week Periods)

Four week period ending	*Luton*	*Cambridge*	*Hornchurch*	*Thurrock*	*Bedford*	*Chelmsford*	*Romford*	*Billericay*	*Brentwood*	*Watford*	*Ipswich*	*St Albans*	*Norwich*
							1944						
Jan 29	7	3	8	10	3	5	5	—	5	7	8	—	27
Feb 26	9	5	22	9	5	1	8	5	3	10	8	2	14
Mar 25	7	3	8	10	3	1	9	3	1	8	5	10	15
Apr 22	5	2	5	4	2	—	3	1	2	8	7	8	26
May 20	10	5	11	2	5	6	6	5	1	5	6	3	22
June 17	14	4	1	5	4	3	5	2	2	2	4	—	14
July 15	7	2	4	7	—	1	2	2	3	5	1	2	7
Aug 12	7	4	4	1	1	1	4	3	—	3	3	4	10
Sept 9	3	3	4	—	3	—	—	3	3	1	4	6	5
Oct 7	4	6	4	4	6	3	3	3	1	1	7	9	15
Nov 4	6	11	2	10	9	1	3	—	4	4	11	6	11
Dec 2	13	14	3	4	6	1	2	1	3	2	1	6	13
Dec 30	5	10	1	2	4	—	—	—	3	2	1	6	10
Total	*97*	*72*	*77*	*68*	*51*	*23*	*50*	*28*	*31*	*58*	*66*	*62*	*189*
							1945						
Jan 27	6	7	3	3	1	3	2	6	2	2	3	1	6
Feb 24	11	6	5	4	1	—	1	—	6	1	3	5	7
Mar 24	9	7	3	2	2	1	1	2	6	2	1	—	1
Apr 21	8	1	1	—	—	—	2	—	5	—	—	1	1
May 29	7	4	9	3	—	1	1	—	3	1	1	1	1
June 16	4	2	11	2	—	—	4	1	—	1	2	—	5
July 14	6	3	3	5	—	—	—	3	—	—	—	1	4
Aug 11	1	4	1	3	—	—	—	5	1	—	2	1	1
Sept 8	3	4	1	5	—	1	1	—	1	2	—	—	3
Oct 6	5	4	1	4	1	1	2	—	1	6	2	1	1
Nov 3	3	1	1	6	2	1	1	1	1	3	1	1	3
Dec 1	4	1	1	4	5	1	—	3	1	2	—	1	5
Dec 29	—	1	3	1	2	3	1	1	—	1	—	2	2
Total	*67*	*45*	*43*	*42*	*14*	*12*	*16*	*22*	*27*	*21*	*15*	*15*	*40*
							Attack Rates per 1,000 (1944 population)						
1944	1·0	0·9	1·0	1·0	1·0	0·7	0·9	0·8	1·1	0·9	0·8	1·5	1·9
1945	0·7	0·6	0·5	0·7	0·3	0·4	0·3	0·6	1·0	0·3	0·2	0·4	0·4
1944 population × 1,000	101	78	82	60	53	33	57	37	26	67	87	41	102

a considerable proportion of the 1945 cases were non-icteric cases notified by one practitioner In 1944 St Albans had small outbreaks in schools, and Norwich had a high incidence in the early part of the year, but in 1945 both places showed a reduced number of cases evenly distributed throughout the year The cases in Norwich were scattered over the city in both years, although during 1944 a few areas showed groups of cases over several months on spot maps kept by the Medical Officer of Health In 1946 an endemic series of cases was notified from eleven towns and in 1947 from twelve Outside the Region, in Wembley, a similar endemic prevalence (Table 13, p 26) followed an epidemic, and in Southend it occurred after the epidemic of 1944 and at the beginning of 1945 In Cambridge, *however, several medical practitioners and headmasters said* that there had been no previous epidemic, so that the endemic type of prevalence cannot be regarded as invariably the aftermath of an epidemic

During the years 1944 to 1947 most of the 72 smaller boroughs and urban districts in the Region reported none or very few cases In 1944 the exceptions were Bishop's Stortford, Bungay, Beccles and Haverhill, where there were epidemics early in the year, and St Ives and Cromer, where there were cases in an endemic series In 1945 Brightlingsea and Witham had epidemics in the autumn (Table 14, p 28)

In rural districts more than half of the villages from which cases were reported had only a single case Between 19 and 33 per cent. of all cases reported from villages were in villages with only one case in the year (Table 9) Epidemics in villages did not persist as in towns (p 27), but in villages as in towns, sporadic cases were common

TABLE 9

Distribution of Cases in Villages

	Year	Number of cases in village							Total
		1	2	3	4	5	6–9	10+	
									Villages
Percentage of villages	1944	51·9	17·9	9·9	6·3	3·7	6·9	3·4	609
	1945	59·0	16·1	10·8	5·1	3·1	3·4	2·5	293
	1946	61·9	17·1	7·4	4·2	1·7	5·4	2·3	286
	1947	63·5	18·6	3·8	6·4	1·9	5·2	0·6	156
									Cases
Percentage of cases	1944	18·9	12·9	10·9	8·4	7·1	17·9	23·9	1,679
	1945	27·9	15·2	15·5	9·8	7·2	11·0	13·4	621
	1946	29·4	16·2	10·4	7·9	4·2	18·7	13·2	603
	1947	33·5	19·6	6·1	13·7	5·1	18·6	3·4	296

GEOGRAPHICAL DISTRIBUTION

In 1944 attack rates above the rate for the Region were registered in three counties in the north-eastern part—East Suffolk, Norfolk and West Suffolk (Table 6, p 15, and Fig 8) Some smaller areas in the northern part of Essex and the western part of the Region also had higher rates The eleven

towns with an endemic prevalence were situated in the southern and western parts of the Region (Fig 9)

In 1945 Norfolk was the county with the highest rate, while East Suffolk had a rate above that for the Region due to a persistence of an epidemic in and around Lowestoft (Table 6 and Fig 8) The Rural Districts of Thingoe and Cosford in West Suffolk had rates above 1 per 1,000 which were due, as high rates in Thedwastre and Cosford in 1944 had been due, to sporadic cases in many villages Epidemics accounted for the other high rates, except for those in Hunstanton, Southend and Benfleet in 1945, where the prevalence was more of the endemic type and attack rates were too low to be considered as epidemic The persistence of a high rate in Norfolk and of a higher rate in its rural districts than in its urban areas was compatible with the idea that the centrifugal spread from London which appeared to have occurred in 1944 reached a further stage in 1945

In 1946 when the incidence in the whole Region rose slightly, there was a definite rise in the rate in Essex from 1 1 and 0 7 per 1,000 in the two previous years to 1 3 per 1,000 The rates in the rest of the Region in the three years were 1 4, 0 6 and 0 5 per 1,000 The high rate in Essex in 1946 was due to high rates in Southend and other urban districts on the south coast (*Southend, 3 6 per 1,000, Benfleet, 2 8, Thurrock, 2 0*, Canvey Island, 1 4, Billericay, 1 4, Brentwood, 1 3, Hornchurch, 0 9) Apart fr[illegible] one rural district Tendring, had a high rate (1 8 per [illegible] ases in many villages throughout the year [illegible] l to that in Essex due to 39 cases in St Neots and 41 cases spread over the other districts In Norfolk, where the rate was equal to that for the Region (0 7 per 1,000), two small towns, Thetford and Sheringham, had 28 and 46 cases during the year Small outbreaks occurred in three villages in other parts of the county In East Suffolk two villages, Wyverstone and Grundisburgh, had outbreaks Over the rest of the Region cases occurred sporadically and 11 towns showed an endemic series of cases The main feature of the distribution of epidemics in 1946 (Fig 10) was the group of towns in south Essex where epidemics followed an endemic prevalence in previous years Epidemics were also reported from the north coast and the north western corner of the Region which had not contributed any in previous years These appeared to represent a continuation of the centrifugal spread suggested by comparison of the 1944 and 1945 distributions

In 1947 Essex again had the highest rate (0 7 per 1,000) and Norfolk had a rate equal to that for the Region (0 4 per 1,000) The distribution of cases in these and other counties was mainly as an endemic series in some towns and as widely scattered cases in villages In Essex three small towns reported outbreaks Wivenhoe had 29 cases in the first three months following 6 cases in November and December of 1946, Brightlingsea had 62 cases, many of them non-icteric, between April and November, and West Mersea had 16 cases in November and December. The map of epidemics in 1947 (Fig 10) shows the situation of these small towns in the eastern part of Essex and indicates how restricted was the area from which epidemics were reported, though there were twelve towns in various parts of the Region where cases occurred in endemic series There were also many sporadic cases in villages (p 17) The findings are compatible with a further stage in the decline of an infection which had spread centrifugally from London over the Region

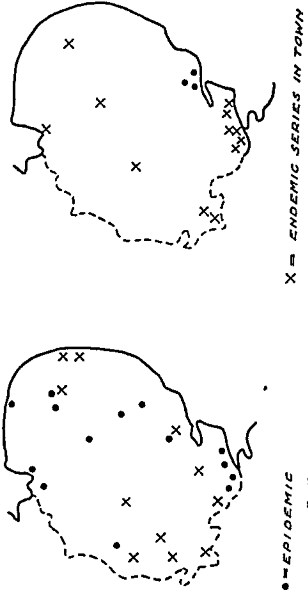


Fig 10 *Geographical Distribution of Epidemics and Towns with Endemic Series (1946 and 1947)*

GEOGRAPHICAL SPREAD OF EPIDEMICS

In 1943 with the assistance of the late Dr B Wood-White, then Medical Officer of Health for East Suffolk, studies were made of the notifications of "jaundice" from schools in the county In Blyth Rural District and Leiston cum Sizewell Urban District there had been an irregular spread from one village school to another (Table 10 (i) and Fig 11) The first schools affected were those of isolated villages in the north then cases occurred in ... the infection spread north ... Visits were paid to the ... n Information given by doctors, particularly by Dr D G Garnett of Leiston showed that the

TABLE 10

Epidemics in Schools (Cases per month)

(i) Leiston Urban District and Blyth Rural District

Place	1942							1943						*Total cases*	*Attack rate (per cent)*
(see Fig 11)	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*June*		
1 Middleton	2	3	7	5	2	—	1							20	30 0
2. Peasenhall*				2	1	3								6	9 6
3 Westleton					2	6	6	9	1					24	37 5
4 Yoxford						2	1	1	1	1				6	5 8
5 Leiston Senior					1	1	1	3	5	10	1	2		24	11 7
6 Leiston Junior							1	4	1	3	2	4	1	16	6 6
7 Knodishall									3	2	7		1	13	20 9
8 Theberton											1	1	4	6	—
9 Darsham											3	10	1	14	29 2

* 3 cases in March 1942

(ii) Stowmarket Urban District and Gipping Rural District

Place	1943												*Total cases*	*Attack rate (per cent)*
(see Fig 12)														
													83	22 4
1 Stowmarket Ar-	2	4	11	[illegible]	[illegible]								25	7 4
Stowmarket Junior			[illegible]		7	12	3						23	10 4
2 Combs Ford					2	1	1						4	4 8
3 Combs				1	1	3	2		3	1			13	24 8
4 Battisford								1	1					
5 Little Finborough									1	2	1		4	—
6 Creeting St Mary						1			1	4	4		10	—
7 Haughley									2				2	—
8 Stowupland														

epidemics had been due to infective hepatitis and that villages had been affected in the order shown by the notifications from schools Only occa ...al cases had been seen in previous years

... of the course of an epidemic ... d of the centrifugal spread to ... 0 (ii) and Fig 12) The cases ... eresting The first two were ... Stowmarket and a girl who ... a boy who attended the ...

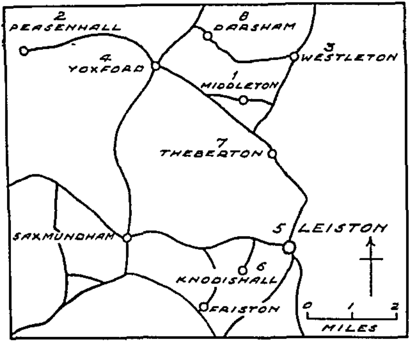


FIG 11 *Spread to and from Leiston* (1942-43)
Numbers refer to Table 10 (i)
Cases occurred in Saxmundham in 1943 and an epidemic in Friston in 1944 (p 27)

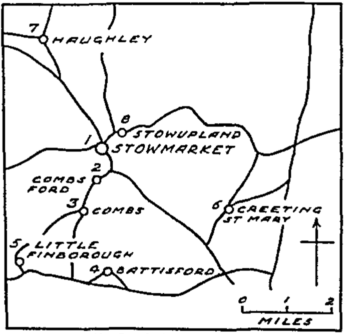


FIG 12 *Centrifugal Spread from Stowmarket*
Numbers refer to Table 10 (ii)

TABLE 11

Dates of Onset of Cases in Battisford

Case No	Onset date	Age and Sex M	F	School	Remarks
1	April 20	12	—	Stowmarket Area	
2	May 17	—	15	None	Worked in Stowmarket
3	June 11	5	—	Battisford	Brother at Stowmarket Area School
4	June 22	—	10		Sister of case 1
5	June 23	—	6		Sister of case 2
6	July 12	—	8		
7	July 19	—	7		Sister of cases 2 and 5
8	Sept 9	—	12		
9	Sept 9	—	11		
10	Sept 9	—	10		
11	Oct 11	7	—		
Little Finborough					
1	Aug 8	12	—		
2	Sept 17	—	9		

worked in a café there A month later there were cases among their brothers and sisters who attended the Battisford school and a month after that children who lived in Little Finborough but went to school in Battisford were affected This sequence of cases indicated a spread by contact and an incubation period of about a month Spread further south occurred in the autumn when a boy in Creeting St Mary fell ill a month after a visit to Stowmarket In autumn the disease also appeared in schools in villages to the north of Stowmarket There were no known cases in Stowmarket at the time but there may have been chronic carriers of the virus or mild or subclinical cases which could have been the source of infection Similar sources might have been present in the northern villages themselves where it was said that there had been cases in adults earlier in the year

TABLE 12

Epidemics in Villages and Towns (Cases per month)

Bungay Urban District, and Wainford and Loddon Rural Districts

	Place (see Fig 13)	1943 May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	1944 Jan	Feb	Mar	Apr
1	Bungay	3	3	11	2	19	20	22	3	5	5	1	1
2	Broome			1	2	1	3	4					
3	Ellingham					3	2	2					
4	Ditchingham				10	7	6	4	1	2	1		
5	Hedenham					4							
6	Earsham						2	3			1		
7	Flixton						2	4					
8	Elmhall St Cross			2									
9	St James						1						
10	St Margaret						1						
11	Ilketshall St Andrews	1		1		2	2	2	1	3			
12	St Margarets				1		4						
13	St Lawrence	1					2			1			
14	St Michaels						1			1	1	2	
15	Mettingham						2						
16	Beccles			6	4		3	1		4	11	2	5

Another example of centrifugal spread was provided in 1943 by reports of cases in Bungay and neighbouring villages made by Dr A Mercier and Dr P G Levick and by schoolteachers (Table 12 and Fig. 13). From Bungay infection spread north-east to Broome and Ellingham, north west to Ditchingham and Hedenham and west to Earsham and Flixton To the south cases occurred during the summer in several isolated villages but the main crop of cases was in October following cases in the Bungay schools in September Infection appeared to have spread through the Area school The town of Beccles to the east down the river Waveney had a succession of cases throughout the year which could not be linked up with the Bungay cases but two children in Mettingham a village between Bungay and Beccles who fell ill in October were probably infected while attending the Area school in Bungay

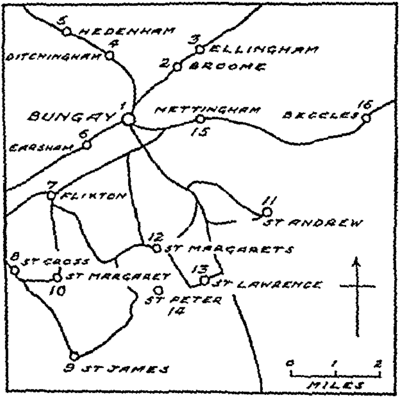


FIG 13 *Centrifugal Spread from Bungay*
Numbers refer to Table 12.

In these three districts there was a centrifugal spread from the main town, preceded in Blyth Rural District by a spread from the north to Leiston the centre for a later dissemination There were irregularities in the spread due sometimes to the escape from infection of places along the roads between affected towns or villages and sometimes to the way in which the infection smouldered in some places during the summer and flared up in the autumn

The widespread distribution of infective hepatitis and its declining incidence in 1944 and later years gave no further opportunities for studying the spread of epidemics, but a similar spread from an epidemic centre probably accounted for epidemics in a number of villages round Lowestoft during these years (Table 15, p 29) and in a few instances an isolated case in a village appeared to have been infected whilst visiting a nearby town where the disease was endemic

EPIDEMICS IN TOWNS

The epidemic in Wembley in 1942 43 has been described by Ford (1943 and 1944) Wembley is outside Region 4, but by the kindness of Dr A G Morrison, the Medical Officer of Health, four weekly returns of the numbers of cases voluntarily reported by doctors up to September 1945 were sent to Cambridge (Table 13) In May 1943 inquiries made in Southend by Dr A Stevenson Logan, the Medical Officer of Health, revealed that an epidemic was in progress Reports from schools and doctors were obtained from January till November when compulsory notification was introduced (Table 13) In both these large urban communities the figures for the early months were obtained retrospectively and are incomplete, but they suffice to show that there were numerous cases in the towns for some months before the peak of the epidemic was reached The rise of the epidemics appeared to be relatively slow suggesting that spread had been by contact

TABLE 13

Epidemics in Towns (Cases in four week periods)

Four-week	*Wembley*				*Southend on Sea*			*Lowestoft*		*Bishop s Stortford*		*Bungay*		
Feb 27		57	2	3	*11*	34	5	6	12	10	–		[illegible]	
Mar 27		31	9	4	*72*	19	14	2	12	12	–		1	1
Apr 24		49	3	–	*77*	6	9	4	17	17	2		–	–
May 22		22	9	–	*96*	11	12	4	7	6	–	1	1	–
June 19		12	6	4	*68*	–	24	6	7	5	–	4	1	–
July 17	1	7	5	–	*40*	5	26	10	10	4	–	11	–	–
Aug 14	4	1	5	2	*11*	3	15	4	3	4	2	1	–	–
Sept. 11	2	11	1	2	*21*	2	7	19	9	3	3	9	–	2
Oct 9	4	21	–	3	*28*	2	10	20	11	5	–	15	–	–
Nov 7	23	16	2		*11*	2	8	22	11	1	–	21	–	–
Dec 5	21	6	1		*12*	3	9	28	10	1	2	18	–	–
Jan. 2	44	5	1		*4*	4	9	21	4	–	–	4	–	–
Total	(99)	340	49	(20)	463	112	152	153	129	78	9	84	13	3
Population	115 000				89 000			28 000		11 000		3 000		

* 1943 figures for Southend are for schoolchildren only
The dates in column 1 are correct for 1943 and approximate for the other years

The same conclusion was reached by Ford (1943) as the result of his field investigations In both towns cases continued to occur after the epidemic and there was a rise in the following autumn Wembley showed an endemic l - of prevalence in 1944 and 1945, which was probably a true reflection ... nterest in

an endemic series of cases in the latter part of 1944 and the early part of 1945 was followed by a rise in the summer of 1945 Some of the cases at that time were infected by soldiers who developed the disease while at home on leave from Holland or Germany During the first eleven months of 1946 the numbers of cases with onset in successive four-week periods were 16, 21, 55, 76, 74, 64, 43, 24, 28 23, 22, 15 A number of these cases were in persons who had been resident in Southend for less than a fortnight before the onset of illness, including some returned prisoners of war and ex servicemen There were 31 such cases scattered evenly over the first seven four-week periods Infection had occurred in these cases before arrival and they represented a considerable importation of infection Nevertheless being just under 10 per cent. of the cases in seven months they did not explain the whole rise in incidence There was a rise at the same time in other urban districts along the north bank of the Thames between Southend and London

In Lowestoft the incidence was high throughout 1944 and 1945 with a rise in the autumn of both years In Bishop's Stortford and Bungay there were only a few cases in the years following an epidemic

The epidemics in large and small towns lasted for several months and had a slow rise and fall After an epidemic the disease became endemic in large towns and practically disappeared from small towns

EPIDEMICS IN VILLAGES

Epidemics in villages were usually of a long straggling type lasting for many months (Table 14) and showing groups of cases at 20- to 40-day intervals (Table 15) There were some more explosive outbreaks, e g at Debenham, Guilden Morden, Swavesey and Brightlingsea (Table 14) and Darsham (Table 10 p 22)

The outbreak at Debenham in 1944 was peculiar in that nine of the cases were in adults It was not possible to find intervening cases linking them with cases which had occurred six months earlier mainly in schoolchildren Milk, water and food were obtained from different sources, and there was no meeting place common to all the cases At Swavesey, similarly, no common source of infection for the first cases could be found, although secondary cases occurred in some families The most probable explanation [illegible]

numerous

A similar explanation appeared necessary to account for the adult cases which occurred in Fen Ditton at the end of June 1943 (Table 15) Investigations were made with the help of Dr H Hartley who supplied data about earlier cases Most of the adults had had no contact with previous cases, but a "Wings for Victory" week at the end of May had been the occasion for several social gatherings and contact with carriers might have occurred at that time

In 1943 in North Lopham and in Debenham spread among schoolchildren and in families accounted for most of the cases The outbreak at Friston in 1944 is a good example of a village epidemic and it will therefore be described in some detail (Table 16, p 30)

In the week ending 11th March 1944 the headmaster of Friston school

TABLE 14

Epidemics in Smaller Towns and Villages (Cases per month)

County	Place	1944 Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total cases
Norfolk	Aylsham	2	5	5	5	5	4	13	3	11	16	2	1	*72*
	N Lopham	4	1	3	–	–	1	5	2	5	5	–	–	*26*
	W Walton	–		1	4	–	1	–	–	–	–	–	–	*6*
	Bessingham*			–	–	–	–	–	3	2	2	–	–	*7*
E Suffolk	Carlton Colville	1	–	1	–	5	1	–	–	–	–	–	1	*9*
	Barnby	–	1	–	1	–	–	–	–	2	2	1	–	*7*
	Kessingland	–	–		–	2	1	–	–	–	7	3	–	*13*
	Mutford	–	–	–	–	1	3	3	3	5	1	1	–	*17*
	Debenham†	1			–	9	1	–	–	–	–	–	–	*11*
	Friston		2	15	6	2	1	–	–	–	–	–	–	*26*
	Walpole		–		–	1	1	2	3	–	1	1	–	*9*
	Somersham	–	–		–	–	–	–	–	3	2	3	2	*10*
	Bricet	–	–		–	–	–	–	2	2	1	2	–	*7*
W. Suffolk	Bildeston	1	–	2	2	1	5	1	1	–	–	1	1	*15*
	Chelsworth	–	–	–	2	4	–	1	1	–	–	–	–	*8*
	Hessett	–	–	1	–	–	–	–	–	4	6	–	–	*11*
Cambs	Melbourne	2	8	2	3	2	5	2	–	2	1	5	1	*33*
	Meldreth		2		1	1	–	7	4	2	5	–	–	*22*
	Guilden Morden		–		–	–		–	–	2	9	–	–	*11*
	Swavesey		–	–	–		–	–	–	–	5	3	–	*8*
Beds	Potton	5	5	3	1	3		–	–	–	–	–	1	*18*
	Lt Barford		1	2	4	1	–	–	–	–		–	–	*8*
	Keysoe		–	–	–	–	–	–	2	3	3	6	1	*15*
Essex	Lt Clacton	2	1	4	3	2	3	–	1	–	–	–	–	*16*
	Rowhedge			–	2	8	3	–	1	1	–	1	–	*16*
	Tiptree				–	–	–	–	–	4	1	2	1	*8*

* Near Lowestoft (Table 13)
† Debenham, 1943 Oct 10 cases, Nov 5 cases Dec 6 cases

County	Place	1945 Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
Norfolk	Reedham[1]	8	1	1	1	–	–	–	–	–	–	–	–	*11*
	Necton[2]	7	2	–	–	–	–	–	–	–	–	–	–	*9*
	Martham	3		3	2	1	2	–	–	1	–	–	–	*12*
	Starston		3	1	1	–	–	–	–	–	–	–	–	*5*
	Goodestone	–	–	5	1	–	–	–	–	–	–	–	–	*6*
	Saham Toney	–			1	–	2	3	1	–	–	–	–	*7*
	Redenhall			–	–	–	–	2	2	2	–	–	–	*6*
	Hoveton				–		–	4	3	4	2	–	2	*15*
	Harleston	–	–			–	–	–	–	3	2	2	1	*8*
	Needham	–	–	–	–	–	–	–	–	2	3	9	3	*17*
E Suffolk	Blundeston*[3]	2	1	2	2	–	–	–		–	–		–	*7*
	Oulton*[4]	5	–	1	2	–		–	–		–	–	–	*8*
Hunts	Alconbury	–	2	6		1	–	–	–	1	–	–	–	*10*
Essex	Frinton	1	3	5	4	2	1	–	–	–	–	–	–	*16*
	Walton	4	2	–	–	3	–	–	–	–	–	–	–	*9*
	Witham	2	–	–	–	–	1	–	–	2	5	4	3	*17*
	Silver End	–	–	–	–	–	–	–	–	3	5	3	–	*11*
	Brightlingsea	2		–	–	–	–	–	1	–	–	19	1	*23*

* Near Lowestoft (Table 13)
[1] Reedham, 1944 Nov 1 case, Dec 2 cases
[2] Necton, 1944 · Nov 2 cases, Dec 1 case
[3] Blundeston, 1944 Nov 1 case
[4] Oulton, 1944 Dec 2 cases

TABLE 15

Epidemics in Villages   Date of Onset of Each Case (Adult cases are shown in italics)

Day of month	1st—9th	10th—19th	20th—end	Total cases (per month)
Fen Ditton				
1943 Feb	–	–	26	1
Mar	*9*	–	30	2
Apr	*6*	16	20 *21*	4
May	4 6 9	11 13	*23* 28 29	8
June	*1* 2 *9*	*14* *19*	*22* *2'* 23 *23* *23*, *25* 25 *29*	13
July	4 *4* 6	*14* 19	*21* 26 23 *23*	9
Aug	–	–	23	1
North Lopham				
1943 Oct	–	–	24 27 29	3
Nov	*1*	–	*21* 22 24 25 25 26	7
Dec	3	16	28 30	4
1944 Jan	1 1	–	–	2
Feb	8	–	–	1
Mar		11 *11*		2
Apr	–	12	–	1
May	–	12	–	1
June	–	*12*	–	1
July	4 4 *4* 7	–	20	5
Aug		*17* *19*	–	2
Sept.	*9* *9*	–	–	2
Oct	*3*	12	23	3
Debenham				
1943 Oct	7	12, 18 18	20 22 *23* 25 29 29	10
Nov	1 1 2 5	11	–	5
Dec	6 8	–	21 24 24 31	6
1944 Jan	5		–	1
Feb				–
Mar				–
Apr				–
May	*1*		*20* *25* *27* *27* *28* *29* *30* *30*	9
June	7			1
Friston				
1944 Feb		15	*21*	2
Mar	3 3 3 3 4 *7* 8 9	10 *13* 13 17	21 22, 25	15
Apr	4 7 *8*	*13* *16* 18	–	6
May	7	–	25	2
June	–		20	1

notified to Dr A G Atkinson then Acting Medical Officer of Health East Suffolk County Council four cases of jaundice and six suspected cases. During the next week a visit was paid to the school with Dr W M Burns Medical Officer of Health Blyth Rural District Council and information was collected there and from patients and their doctors—Dr D G Garnett and Dr A Burlingham of Leiston Dr E A Collins and Dr D W R Richardson of Saxmundham and Dr Norah Acheson of Aldeburgh There were no cases of hepatitis in the surrounding district at the time although there had been epidemics in previous years (Table 10 (i) p 22, and Fig 11, p 23)

The symptoms and signs in the recent cases were typical of infective hepatitis The children affected were in several classes at school, came from different families and lived in houses scattered over the village or

in one instance on an outlying farm There had been cases in the village in December 1944 and February 1945, all were in schoolchildren except one in a schoolteacher (Case 2) It appeared that infection was spread through the school It was arranged that any schoolchildren who vomited should be excluded for a week and then allowed to return if well Children who developed jaundice were to be excluded for a fortnight from the onset of symptoms or till recovery if that were longer

Locality and population The village is 2½ miles south east of Saxmundham, the same distance south west of Leiston and 2 miles north of the River Alde The Aldeburgh-Saxmundham road runs through it, and the school and some houses are on the road Most of the houses are scattered along narrow lanes off the main road The adults for the most part work on farms, a few work in Leiston at a factory or elsewhere Buses run to Saxmundham and to Leiston, and adults go there to shop occasionally Water supplies are derived from many shallow wells Pail privies are universal

The course of the epidemic (Table 16) The first case in a schoolchild occurred in the middle of December 1943 shortly after she had returned to school after recovering from pertussis It seemed possible that there had been one or more missed cases during January because the first two cases in the outbreak were a young girl, who was a cousin of the December case,

TABLE 16

Epidemic at Friston in 1944

Case No	*Date of onset*	*Age and Sex* M	F	*Household*	*School Class*
1	Feb 15		12	A	I
2	21		30	B	(3)
3	Mar 2		14	C	1
4	3	13		D	1
5	3	12		E	1
6	3	13		F	1
7	4	7		G	3
8	7		19	H	2
9	8		9	I	3
10	9		12	J	2
11	10	7		K	3
12	13	18		L	–
13	13		8	M	3
14	17	6		I	3
15	21	9		N	3
16	22		7	O	3
17	25	14		P	1
18	Apr 4	6		Q	3
19	7		3	G	–
20	8	16		G	–
21	13	18		P	–
22	16	33		D	–
23	18	12		P	3
24	May 7	11		G	1
25	25	5		R	3
26	June 20	11		R	2

and the teacher of the class attended by the December case No suspicious illness had occurred in schoolchildren or in the members of the three households concerned, but asymptomatic infections might have occurred There were fifteen cases in March of whom fourteen attended the school and might have been infected there The exception (Case 12 of Table 16) was a youth who lived and worked in a labour camp at Tilbury He came to his home in Friston on 5th and 26th February and stayed for 2 days on each occasion There were no known cases at the labour camp nor in his family—father, mother and adult sister His illness began at the camp on 13th March with anorexia, nausea, headache and shivering He came home on the 18th and was jaundiced on the 21st The times from his visits to Friston till his first symptom were 35 to 37 and 14 to 16 days It seemed probable that he was infected by a carrier at Friston

In April there were six cases, five in households where there had been a case in March Two cases occurred in May, one (Case 24) infected at home and

TABLE 17

Attack Rates in Friston

In the Parish

Age	*No*	*Cases*	*Attack rate (per cent)*
0—4	32	1	3 1
5—14	60	20	33 3
15+	329	5	1 5
All ages	*421*	*26*	*6 2*

In those exposed to a case in the family

Age	*No*	*Cases*	*Attack rate (per cent)*
0—4	4	1	—
5—9	4	0	—
10—14	8	3	—
15+	39	3	7 7
All ages	*55*	*7*	*12 8*

In Schoolchildren

Age	*No*	*Cases*	*Attack rate (per cent)*
5—9	28	9	32 1
10—14	37	11	29 7
5—14	65	20	30·4

Sex	*No*	*Cases*	*Attack rate (per cent)*
Male	41	13	31 7
Female	24	7	29 2
Both sexes	*65*	*20*	*30 4*

the other (Case 25) at school The latter infected his brother who fell ill in June

Cases in households Eighteen households were affected, thirteen had a single case, one (P) had three cases and another (G), four cases The days between the onset of the cases in the five families with more than one case were —household D (Table 16), 44 days, G, 34, 1, 29 days; I, 9 days, P, 19, 5 days, R, 26 days These intervals suggested case to case infection with an incubation period of 20 to 40 days The two cases in household I might both have been infected at school

Cases in schoolchildren The attack rates in the three classes, in two age groups and in the two sexes were not significantly different (Table 17) The findings of a rate as high in the 10 to 14 age group as in the 5 to 9 age group is usual in infective hepatitis (p 13)

The method of spread of infection The secondary attack rate at all ages in persons exposed to a case at home was twice the attack rate in the village as a whole, and the attack rate in adults exposed at home was five times that in all adults in the village (Table 17) These findings emphasized the importance of contact in the spread of infection, but it was noted in this epidemic that the persons affected were not the mothers who looked after the children during their illness but a father or older or younger siblings Similarly children who appeared to have been infected at school had not all had particularly close contact with the child who fell ill a month earlier It appeared either that relatively slight contact had transmitted infection or that there were carriers with whom the later case had had close contact, or more probably that infection had been transmitted by both slight contact and by carriers

Spread by milk, water or food was unlikely owing to the distribution of cases among the customers of two independent milk retailers, the use of many separate wells and the purchase of food from different sources The school water came from a satisfactory source and bacteriological examination showed no evidence of faecal contamination

Other diseases in the village Pertussis was prevalent in the autumn of 1943, as also was "influenza" Rubella occurred at that time and also during the hepatitis epidemic Case 20 had a typical rubella rash and enlarged lymph nodes on 8th April, 3 days before jaundice appeared His brother (Case 7) developed rubella on 28th April

Conclusion Cases occurred before the apparently explosive outburst in March, and infection spread at first in the school Subsequently there were instances of case to case spread in families, with intervals of 20 to 40 days between cases The outbreak was to be explained on the basis of contact spread in which carriers might have played a part, and not by the ingestion of milk, water or food contaminated before distribution

Similar epidemics in villages in England have been described by Pickles (1939) and Newman (1942) and others, and in Germany by von Bormann *et al* (1943) Case to case infection was traced in many instances, and spread of infection by other means than contact was excluded or considered most improbable Von Bormann and his co authors found that most of their epidemics arose *de novo*, and suggested that the virus must be widespread in non epidemic times, causing atypical illness or subclinical immunizing

infections because when an epidemic occurred the incidence was greater in the younger age groups

In villages in Region 4 even more than in the small towns, an epidemic was usually followed by a period with very few cases or none. Pickles (1939) noticed long intervals between epidemics in Wensleydale, and Lisney (1944) found that villages in Leicestershire which had had epidemics between 1936 and 1942 were not affected during the severe epidemic in the winter of 1942-43. These observations suggest that an epidemic has an immunizing effect

EPIDEMICS IN RESIDENTIAL AND DAY NURSERIES

In several epidemics in nurseries there was a straggling series of cases at 20- to 40 day intervals for months (Table 18). In most of the nurseries

TABLE 18

Epidemics in Residential and Day Nurseries. Date of Onset of Each Case (Cases among nurses are shown in italic)

Day of month	*1st—9th*	*10th—19th*	*20th—end*	*No of cases per month*		
				Nurses	*Children*	*Total*
Day Nursery, Welwyn						
1943 Mar	*2*		*26* *28*	3		3
Apr	*7*	13		1	1	2
May		*13*		1		1
June	*2* *2*	17		2	1	3
Residential Nursery, Godmanchester						
1943 June		*12*	*26*	2		2
July	1 1 2		*23* *25* *26*	3	3	6
Aug	4				1	1
Residential Nursery, Godmanchester						
1943 Mar		13 15			2	2
Apr	4				1	1
May		17	22		2	2
June			23		1	1
July	8	18			2	2
Aug			23 26		2	2
Sept		16 *16* 19	*23*	2	2	4
Residential Nursery, Bengeo						
1944 Sept		*12*		1		1
Oct		*11*		1		1
Nov	*3*			1		1
Dec						
Jan	*8*	*16*		2		2
Feb		*19*	*23*	2		2
Mar *		11			1	1
Apr *		16			1	1
Convalescent Home, Abbots Langley						
1944 June			24		1	1
July		13			1	1
Aug	*9* *9*	*11* *15*	*23* 29	5	1	6
Sept			23 26		2	2
1945 Jan			21 *23*	1	1	2
Feb	7				1	1
Mar	3				1	1

* Three non-icteric cases in children with onset 19th March, 11th and 23rd April, and two children with bilirubinuria without symptoms on 21st and 26th March (Pollock 1945a)

there was a high incidence in nurses, who were mainly girls aged 16 to 20, and a low incidence in children aged 2 to 5 years Case to case infection among nurses would have accounted for most of the cases, but subclinical attacks in children might also have been responsible Pollock (1945a) obtained biochemical evidence of such attacks in the nursery at Bengeo, which had had a straggling series of cases in nurses and a few cases in children Franklin (quoted by Cullinan, 1939) noted transient biluria in children exposed to cases of infective hepatitis

The dates of onset of cases in the children's convalescent home at Abbots Langley provide an example of an extended epidemic among children aged 3 to 12 years Several of the nurses who fell ill in August had had no contact with the only known case in July No illness suggestive of non icteric hepatitis could be found in the carefully kept records It appeared that subclinical cases might have been responsible for the infection of the nurses Foam tests were performed daily on the urine of children at the end of September and the beginning of October, and positive results were obtained only in children who became jaundiced A more sensitive test for biluria might have given evidence of subclinical hepatitis at that time Another possible explanation of the infection of the nurses is that those who were in contact with the July case and developed hepatitis in August were infectious in July soon after the contact and infected other nurses then (p 36)

The observations in nurseries suggest that in children under 5 years of age infective hepatitis is often asymptomatic or mild These observations also provide evidence to support the idea that immunity in adults may be the result of subclinical attacks in early life

INCUBATION PERIOD

Pickles (1939) agreeing with the suggestion made by Booth and Okell (1928) of a 20 to 40-day incubation period, estimated the incubation period in epidemics in Wensleydale at 25 to 35 days The most reliable method of estimating an incubation period is as Pickles urged, to observe the date of onset in cases where illness follows a short, single exposure to infection Such opportunities are not common, but the following cases provide two instances

Case 1 A married woman aged 29 lived in the village of Wix in Essex where [illegible] family [illegible] Suffolk [illegible] feeling [illegible] became jaundiced on 12th July

Case 2 A girl, aged 15 the niece of Case 1 lived at Woolverstone where there had been no cases of jaundice for some years She bicycled daily to work in Ipswich at an office where *there had been no* cases of jaundice On 31st July, 25 to 27 days after her aunt s visit she had a sore throat and severe frontal headache The sore throat persisted till 5th August when she was feverish and lost her appetite On 12th August she became jaundiced

Case 3 A girl aged 10 niece of Case 1 and sister of Case 2 lived in Woolverstone and attended the village school On 9th August 34 to 36 days after her aunt s visit she lost her appetite and her conjunctivae were jaundiced

These observations are compatible with Pickles' estimate of an incubation period of 25 to 35 days Further evidence that the incubation period is of that order is provided by the frequency of 20- to 40 day intervals between successive cases in contacts during epidemics (Table 15, p 29 and Table 18) and by the time distribution of secondary cases in families (McFarlan, 1945b).

where fifty per cent. of all secondary cases commence between 20 and 40 days after the first case No satisfactory evidence of a shorter incubation period has come to notice, though some authors have suggested it Selander (1939) suggested a longer incubation period of 2 or 3 months, but many of the patients on whom his estimate was based were diabetics and it is possible that he was dealing with homologous serum hepatitis There is much evidence that the incubation period in groups of persons who have received [illegible]

of the cases in these groups have had incubation periods of less than 60 days, but the majority have fallen between 60 and 100 days Although an increase in dose may reduce the incubation period (Bradley, Loutit and Maunsell, 1944), it has not been shown that the period is reduced to the 20 to 40 days of infective hepatitis Thus it appears that the incubation period is a differential character between infective hepatitis, where it is short (20 to 40 days), and serum hepatitis where it is longer (60 days or more)

Experimental human transmission of infective hepatitis has given some results which are compatible with the short incubation period and others which suggest a longer period (see pp 124, 122) There is only one record of a long incubation period (61 to 71 days) following the ingestion of faecal materials, and in that instance (Neefe and Stokes, 1945) the ingested well water may have contained only very small quantities of virus Long incubation periods have however been frequent following parenteral inoculation of serum from cases of infective hepatitis In view of the experiments of Havens *et al* (1945) showing short incubation periods following parenteral as well as oral administration of serum from cases of infective hepatitis, the prolonged incubation periods in other experiments cannot be attributed solely to the parenteral route Experiments by Oliphant (1944b) suggest another explanation He found that serum from a case of infective hepatitis, given parenterally produced hepatitis in four of eleven persons who had not previously had hepatitis The incubation periods were from 85 to 106 days The same serum failed to produce hepatitis in ten subjects who had previously had homologous serum hepatitis This was interpreted as a demonstration of cross-immunity between infective hepatitis and homologous serum hepatitis However, since other experiments (p 137) have demonstrated an absence of such immunity, it may be suggested that the serum although derived from a case of infective hepatitis in fact contained the homologous serum hepatitis virus Some at least of the other sera from cases of infective hepatitis which produced long incubation periods may also have contained the homologous serum hepatitis virus

Findlay, Martin and Mitchell (1944) suggested that homologous serum hepatitis in British troops in West Africa behaved epidemiologically like infective hepatitis and had a short incubation period From the published data it is clear that infective hepatitis was prevalent among these troops and that almost all of them had been inoculated with yellow fever vaccine prepared in England In these circumstances the mere record of previous yellow fever vaccination is insufficient to establish a diagnosis of serum hepatitis Many of the findings are most easily explained by assuming that many of their cases were due to the virus of infective hepatitis spreading in its usual fashion among men who had received yellow fever vaccine These considera-

tions apply also to the claim (Findlay and Martin, 1943) to have transmitted homologous serum hepatitis with *nasopharyngeal washings* The incubation periods in their cases were 28, 30 and 50 days, and the two shorter ones are suggestive of infective hepatitis However, the failure of other workers to *transmit* infective hepatitis with nasopharyngeal washings makes it difficult to assess the significance of Findlay and Martin's results

These arguments support the conclusion reached by MacCallum (see p 135) that hepatitis transmitted by serum may be either infective hepatitis or homologous serum hepatitis It has to be remembered that the blood of a case of infective hepatitis *may contain the homologous serum hepatitis* agent and also that persons who have received inoculations of serum may contract infective hepatitis Until it is possible to differentiate infective hepatitis and homologous serum hepatitis by laboratory tests these two explanations of apparently anomalous incubation periods should be considered as alternatives to a conclusion that short and long incubation periods are equally common in infective hepatitis and in serum hepatitis Although exceptions occur and an explanation of them would be both interesting and important, the incubation period of infective hepatitis can be accepted as being about 25 to 35 days and the incubation period of serum hepatitis as about 60 to 150 days

DURATION OF INFECTIVITY

The infectivity of cases in infective hepatitis is greatest in the early stages of the illness The time distribution of secondary cases in families suggests this and suggests also that the period of infectivity is short (McFarlan, 1945b) Secondary cases in hospital staff are more common in surgical wards where patients are often admitted in the pre-icteric stage than in medical wards where they are usually jaundiced on admission The instance of infection *following* a short, and only possible exposure (p 34) suggested that Case 1 was infectious between 6 and 8 days before jaundice developed, and Booth and Okell (1928) made a similar observation of apparent infectivity 6 days before jaundice *developed Pickles (1939) records an instance of* infectivity 5 days after the onset of illness It does not follow, of course, that every case is infectious for 6 days before and 5 days after the onset of symptoms, indeed individual cases appear to be infectious only for a short time Pickles has found that it is safe to presume an absence of infectivity [illegible] cases *a relatively short* period of infectivity in the pre-icteric stage [illegible] occasional cases may be infectious earlier or later

A few observations have suggested that there may sometimes be another period of infectivity early in *the incubation period*, that is 20 to 40 days before the onset of symptoms

(1) An officer in the *U.S.A.A.F developed* infective hepatitis early in January 1944 3 days after a nurse in an isolation hospital had fallen ill with the same disease The officer had not had any contact with a case of the disease but he had frequently met the nurse during the 20 to 40 days before they fell ill She was at that time nursing several cases of infective hepatitis *in hospital*

(2) An officer in the British Army was admitted to hospital in January 1944 with infective hepatitis His sister fell ill at home in London at the same time She had had *no known contact with a case* but had been in contact with her brother during his leave 3 to 4 weeks before they *fell ill There had been several cases among the*

ship
had
his
ister

idice
olice
cases
emic

The usual explanation of two practically simultaneous cases of an infectious disease with a long incubation period is that both have been infected from a common source but these four instances could equally well be explained by supposing that the case with a known contact was infectious a few days after exposure and infected the second case at that time Some of the groups of cases which occurred during epidemics in villages and nurseries (Tables 15, p 29 and 18 p 33) might have had a similar explanation as might some of the cases in an institution for mental defectives during an epidemic which will now be described

EPIDEMIC OF INFECTIVE HEPATITIS IN AN INSTITUTION

Studies of an outbreak of infective hepatitis lasting from February to July 1944 at the Royal Eastern Counties Institution Colchester were made possible by the help and co operation of the Medical Superintendent Dr F D Turner and of the medical nursing and laboratory staffs Biochemical and haematological observations have been reported (Pollock 1945b and p 70 Miles p 84)

The population at risk The 573 mental defectives in the Institution were distributed in five houses A B G M and N which had respectively 115, 115 17 296 and 30 inmates House N contained children of both sexes Houses A and G older persons of both sexes whilst B and M contained older females only Three houses A M and N formed separate communities and contacts between inmates of different houses were few The nurses slept in rooms off the wards and had no contact with the nurses of other houses Houses B and G were similarly separate as regards patients but nurses from B did relief duty in G A few patients in each house (Table 19) had contact with patients from other houses when they attended the school held in the dining hall of House B It will be shown that contact in the school played a part in the dissemination of infection Medical officers and senior members of the nursing staff visited all the houses but it is unlikely that they spread infection

TABLE 19

Houses of Children Attending the School

House	A	N	B	G	M	Total
No attending school	10	14	6	3	5	38
No of these with hepatitis	8	8	3	2	0	21

tions apply also to the claim (Findlay and Martin 1943) to have transmitted homologous serum hepatitis with nasopharyngeal washings The incubation periods in their cases were 28, 30 and 50 days and the two shorter ones are suggestive of infective hepatitis However the failure of other workers to transmit infective hepatitis with nasopharyngeal washings makes it difficult to assess the significance of Findlay and Martin's results

These arguments support the conclusion reached by MacCallum (see p 135) that hepatitis transmitted by serum may be either infective hepatitis or homologous serum hepatitis It has to be remembered that the blood of a case of infective hepatitis may contain the homologous serum hepatitis agent and also that persons who have received inoculations of serum may contract infective hepatitis Until it is possible to differentiate infective hepatitis and homologous serum hepatitis by laboratory tests, these two explanations of apparently anomalous incubation periods should be considered as alternatives to a conclusion that short and long incubation periods are equally common in infective hepatitis and in serum hepatitis Although exceptions occur and an explanation of them would be both interesting and important the incubation period of infective hepatitis can be accepted as being about 25 to 35 days and the incubation period of serum hepatitis as about 60 to 150 days

DURATION OF INFECTIVITY

The infectivity of cases in infective hepatitis is greatest in the early stages [illegible]

patients are often admitted in the pre icteric stage than in medical wards where they are usually jaundiced on admission The instance of infection following a short and only possible exposure (p 34) suggested that Case 1 was infectious between 6 and 8 days before jaundice developed, and Booth and Okell (1928) made a similar observation of apparent infectivity 6 days before jaundice developed Pickles (1939) records an instance of infectivity 5 days after the onset of illness It does not follow, of course that every case is infectious for 6 days before and 5 days after the onset of symptoms, indeed individual cases appear to be infectious only for a short time Pickles has found that it is safe to presume an absence of infectivity a fortnight after the onset of symptoms provided that the patient has clinically recovered Newman (1944) presented evidence of apparent infectivity during a relapse 2 months after a first attack It appears that there is in most cases a relatively short period of infectivity in the pre-icteric stage but that occasional cases may be infectious earlier or later

A few observations have suggested that there may sometimes be another period of infectivity early in the incubation period that is 20 to 40 days before the onset of symptoms

[illegible] infective hepatitis early in January 1944 [illegible] same disease [illegible] equently met [illegible] time nursing

[illegible] ry 1944 with [illegible] She had [illegible] other during [illegible] among the

troopship
She had
during his
his sister
and parents at their house There had been cases in the officer s unit previously

(4) Btesh (1944) recorded that a police sergeant in Palestine developed jaundice at Haifa 4 weeks after being transferred from Athlit A few days later a police constable living in the same barracks developed jaundice There had been no cases of jaundice in the Haifa barracks before these cases but there had been an epidemic at Athlit

The usual explanation of two practically simultaneous cases of an infectious disease with a long incubation period is that both have been infected from a common source, but these four instances could equally well be explained by supposing that the case with a known contact was infectious a few days after exposure and infected the second case at that time Some of the groups of cases which occurred during epidemics in villages and nurseries (Tables 15, p 29 and 18, p 33) might have had a similar explanation, as might some of the cases in an institution for mental defectives during an epidemic which will now be described

EPIDEMIC OF INFECTIVE HEPATITIS IN AN INSTITUTION

Studies of an outbreak of infective hepatitis lasting from February to July 1944 at the Royal Eastern Counties Institution, Colchester, were made possible by the help and co operation of the Medical Superintendent, Dr F D Turner, and of the medical nursing and laboratory staffs Biochemical and haematological observations have been reported (Pollock, 1945b and p 70, Miles, p 84)

The population at risk The 573 mental defectives in the Institution were distributed in five houses, A B, G M and N, which had respectively 115, 115, 17 296 and 30 inmates House N contained children of both sexes, Houses A and G older persons of both sexes, whilst B and M contained older females only Three houses, A M and N formed separate communities and contacts between inmates of different houses were few The nurses slept in rooms off the wards and had no contact with the nurses of other houses Houses B and G were similarly separate as regards patients, but nurses from B did relief duty in G A few patients in each house (Table 19) had contact with patients from other houses when they attended the school held in the dining-hall of House B It will be shown that contact in the school played a part in the dissemination of infection Medical officers and senior members of the nursing staff visited all the houses, but it is unlikely that they spread infection

TABLE 19

Houses of Children Attending the School

House	*A*	*N*	*B*	*G*	*M*	*Total*
No attending school	10	14	6	3	5	38
No of these with hepatitis	8	8	3	2	0	21

Some patients had visitors from time to time Others went into Colchester for a few hours with relatives or (from House B) daily to do domestic work Neither visitors nor visits to Colchester appeared to have introduced infection At the time of the epidemic there were very few cases of infective hepatitis notified from Colchester, though there had been a fair number in the winter of 1942-43 according to information received from doctors Nurses also visited the town and went on leave from time to time, but none who developed jaundice knew of any contact with a case outside the Institution and all might have been infected by cases on their wards

Cooked food was supplied from a central kitchen to all the houses for the patients and to some houses for the nurses In the other houses the nurses cooked their own food which was supplied from the same stores The water supply was the piped town supply No cases of jaundice occurred among the kitchen staff The variation in incidence and in the time of occurrence of cases in different houses made it most improbable that the infection was spread by contaminated food or drink

The Institution had its own laundry which dealt with all the soiled linen None of the laundry staff or patients who worked there developed jaundice

Symptomatology Satisfactory histories and examinations were obtained in 5 nurses and 8 higher grade patients The symptoms, signs and laboratory findings were typical of infective hepatitis

The majority of the cases were in mental defectives who could not give any account of their symptoms and in whom clinical examination was difficult or impossible However, the nurses reported every patient who vomited, or passed dark urine (or had "stained sheets" if incontinent), or became febrile Temperatures were taken only if any alteration in behaviour suggested that the patient was unwell Where possible these patients were examined Their urine was tested for bilirubin on at least 3 days by Hunter's test (Mr A Lee kindly performed many of these tests in the laboratory at the Institution) A bromsulphalein test of liver function and a differential white count were performed on most patients, and in many of them total serum bilirubin and "1 minute Van den Bergh percentage" (Pollock, 1945b and p 70) were estimated

In 65 of these low-grade p[illegible] of jaundice made the diagnosis complete [illegible] a doubtful tinge of jaundice in their [illegible]e cases of infective hepatitis on account of positive Hunter's tests The serum bilirubin in one of these was estimated and found to be 2 1 mg per 100 ml Four other patients were never jaundiced One of them, who was incontinent, had "stained sheets" and a serum bilirubin of 2 5 mg per 100 ml The other 3 had positive Hunter's tests and 1 in whom venepuncture was possible had a serum bilirubin of 0 7 mg per 100 ml and a bromsulphalein retention of 15 per cent These 4 patients were considered to be cases of infective hepatitis Thus in the total of 85 cases including the 5 nurses, there were only 4 who definitely did not become jaundiced (4 7 per cent.) If the 3 with doubtful jaundice are included the percentage of non-icteric cases becomes 8 3

Satisfactory information about the interval between the date of the first abnormality and the onset of jaundice was obtained for 60 cases In 15 jaundice was the first abnormality noted The absence of complaint of pre icteric symptoms in as many as 25 per cent of the patients was probably

the result of their low mental category In 37 (61 7 per cent) the interval between the first abnormality and the appearance of jaundice was from 1 to 7 days in 7 it was from 8 to 12 days and in 1 it was 18 days

Most of the cases were mild Only 1 patient gave rise to anxiety on account of deep jaundice and drowsiness but he made a clinical recovery In 38 cases the degree and duration of jaundice were noted Jaundice was slight in 11 moderate in 24 and marked in 3 It lasted for 7 days or less in 11 between 8 and 14 days in 22 and more than 14 days in 5

One patient had a relapse 25 days after the onset of symptoms and a week after jaundice had disappeared She also made a satisfactory clinical recovery

Two deaths occurred in patients who had had infective hepatitis

(1) An epileptic boy aged 4 developed anorexia and irritability on 29th May Two days later slight jaundice was noted The jaundice faded but status epilepticus [illegible]

[illegible] May shortly after recovering from [illegible] The depth of jaundice was greatest [illegible] and had disappeared by 8th June [illegible] as no recurrence of jaundice and [illegible] Hunter s test Bronchopneumonia [illegible] This patient had had jaundice in [illegible] contacts of 7 cases of hepatitis in patients who had received convalescent measles serum (Propert 1938)

Observations in suspects and contacts Twenty two patients were seen on account of vomiting fever or dark urine In 15 of them repeated Hunter s tests showed the absence of bile from the urine Serum bilirubin was estimated in 9 in 8 the level was 0 75 mg per 100 ml or less in 1 readings of 0 5 and 1 1 mg per 100 ml were obtained In 4 of these patients the total and differential white counts were normal Clinical examination where possible and subsequent observation of the 22 patients gave no reason to suppose that they were suffering from hepatitis Vomiting occurs not infrequently among mental defectives owing to their eating too much or swallowing noxious objects Vomiting had been occurring in the Institution during 1943 and *Shig sonnei* was isolated from rectal swabs in some of the cases This epidemic had died down before hepatitis became prevalent and stools examined from several of the patients with hepatitis yielded no organisms of the enteric food poisoning or dysentery groups There was no reason to connect these suspect cases with one another as they were in many different wards and occurred sporadically during the hepatitis epidemic Apart from respiratory infections in March there was no infectious disease prevalent at the time of the hepatitis epidemic

Hunter s tests were performed on the urine of 28 contacts 19 dormitory contacts in House M were tested three or four times a week for 3 weeks following the fourth case of hepatitis there in March One positive was obtained on 1st May in a girl (1) who was then found to be jaundiced Tests on her urine on 25th and 28th April had been negative 9 persons 3 each in Houses A B and G were tested three or four times a week for 3 weeks following contact with cases in their house and at the school Two positive results were obtained One girl (2) had negative tests on 9th and 10th May and a positive on 11th May She was jaundiced on 12th May The other girl (3) had negative tests on 8th 10th 11th and 12th May a weak positive on 13th May and a strong positive on 15th May Her urine was dark on 8th May but no jaundice or clinical

abnormality was found on 10th or 12th May On 15th May there was marked jaundice of the conjunctivae and slight jaundice of the face and chest. Four serum bilirubin estimations at weekly intervals were made on 6 of these 9 subjects. Five of them gave results within normal limits throughout, the sixth was the girl (3) already mentioned in whom the results were 0 4, 0 15 and 6.1 mg per 100 ml on 4th, 10th and 15th May

These tests were undertaken in an attempt to detect liver damage without symptoms or before symptoms developed They were unsuccessful, but daily examinations of a larger number of contacts might have revealed transient liver damage in a few cases Hunter's test is frequently positive in patients with symptoms before jaundice has appeared Owing to the lack of symptoms in the mental defectives it was hoped that this test would be useful for the early detection of cases It did not prove to be so in the case of girls (2) and (3) in whom frequent examinations were made, there was a gap in the examinations of girl (1) at the critical time Further experience is needed to decide whether the test will detect cases before symptoms develop

The sequence of cases The straggling series of cases in Houses M and B (Table 20 and Fig 14) contrasts with the more explosive outbreaks in the other houses In order to see how far the spread of infection could be explained on the basis of case to case infection with an incubation period of 20 to 40 days it is necessary to study the cases in detail

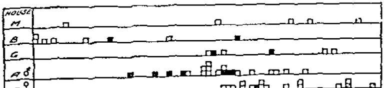


FIG 14 *Epidemic in a Mental Institution Cases by day of onset from March 24th to May 30th*

TABLE 20

*Cases by Houses and Weeks**

Week ending	Feb 26	March 4	11	18	25	April 1	8	15	22	29	May 6	13	20	27	June 3	10	17	24	Total
House M	2	–	–	1	–	1	–	–	–	–	1	–	2	–	1	2	–	–	10
B	–	1	–	1	2	2	2	–	1	–	1	–	–	–	–	1	–	–	11
G	–	–	–	–	–	–	–		–	2	1	1	–	2		–	–	–	6
A	–	–	–	–	–		–	1	2	7	11	8	5	3	1	–	–	–	38
N			–				–	–	1	–	3	7	3	1	2	–	–		17
																			82
Total	2	1	–	2	2	3	2	1	4	9	17	16	10	6	4	3	–	–	

* Cases in nurses are included in this Table
Three cases occurred in House M during July and August (Table 21)

After the cases of hepatitis following measles serum in 1937, none was recorded in the Institution until 1943 In 1942 cases occurred in a completely separate branch and since a special watch for cases was kept, it is practically certain that none occurred in the Institution itself in 1942 and 1943 until December when a woman aged 27 fell ill on the 22nd She was in House M and had returned there only 15 days previously from an isolation hospital where she had been for some weeks with scarlet fever It is probable that she was infected in the isolation hospital although no contact with a case of jaundice could be traced No cases or suspects could be found in the records of House M following this case until 24th February when the first two cases of 1944, M1 and M2 occurred (Table 21)

TABLE 21

Dates of Onset of Cases in Different Dormitories of House M

Dormitory	Feb	Mar	Apr	May	June	July	Aug
B	24, 24	17 30	30	—	5	—	—
P	—	—	—	15	—	—	—
C	—	—	—	19	—	—	—
L	—	—	—	29	5	8 14	1

These patients, M1 and M2, slept in a different dormitory (B), from that of the woman who fell ill in December, and their contact with her could only have been casual in the corridors The next cases, M3 and M4, were women also in dormitory B, and they fell ill 22 and 35 days after M1 and M2 M4 was a friend of one of the earlier cases and had visited and kissed her while she was isolated There were intervals of 31 days between M4 and M5 and 37 days between M5 and the next case in this dormitory In May there were no cases in dormitory B but cases M6 and M7 occurred in two other dormitories, P and C, where they were unique, and had probably been infected by casual contact with M1 and M2 Dormitory L had five cases, M8 to M12, the first of which was in May the interval between M8 and M9 was 7 days, between M9 and M10 33 days, between M9 and M11 39 days, and between M10 and M12 25 days This series of cases among the older and relatively high-grade girls of House M resembles the serial infections found in families

House B The first case, B1 occurred on 29th February, and no connection could be traced between it and the February cases, M1 and M2 B2, a nurse, was the next to develop jaundice, 18 days later She might have been infected by B1 or from a common source The next six cases, B3 to B8, were patients who fell ill between 24 and 39 days after B1 B8 had had "influenza" and had been nursed in the same ward as B1 when the latter had just developed hepatitis Two further cases, B9 and B10, occurred 12 and 26 days after B8, or 27 and 41 days after B3 Here again the 20 to 40-day intervals between cases were unmistakable The prevalence of "influenza" at the time of the first case might have assisted the spread of infection

The School The first two cases among schoolchildren, S1 and S2, were cases B1 and B8 already mentioned The interval between the onsets was 39 days Four of the next five schoolchildren to contract jaundice, S3 to S7 lived in House A, and the fifth in House N, they were the first cases ,

these houses Their illnesses began 43, 48, 51, 53 and 54 days after S1, too late therefore to have been infected from that source They had no contact with S2, who did not attend school after 29th February The most likely explanation was that there was a carrier in the school who was infectious between 20 and 40 days after the onset of S1 Missed cases were unlikely, since the symptoms in all children absent from school at the time were exclusively respiratory There were cases of infective hepatitis in House B at the appropriate time for infection, contact between them and the schoolchildren was improbable, but it is conceivable that infection was spread by fomites or dust in the schoolroom, which was also used as the dining-room for House B

Following the cases S3 to S7, the school was closed on 24th April Twelve more cases, S8 to S19, occurred between 17 and 34 days after the first of the batch, S3, and between 6 and 23 days after S7 Two later cases S20 and S21, schoolchildren in House N, commenced 25 and 30 days respectively after S6, who was also the first case in House N

The sequence of cases in schoolchildren again suggests an incubation period of 20 to 40 days and a building up of epidemic potential by cases and perhaps by carriers as the cause of the rather explosive main batch of twelve cases

House G The first three cases, G1 to G3, occurred between 28th April and 1st May G2, who fell ill on 29th April, attended the school and was probably infected there, but G1 and G3 had no contacts outside the House Infection might have been introduced from House B by one of the nurses on relief duty, but since none of them had any symptoms it can only have been by a healthy carrier Another possibility is that G2 was infectious at the beginning of his incubation period, about 19th March to 9th April, and it is perhaps significant that he was away from school with a cold from 20th to 24th March He, or G1 or G3, presumably infected the two later cases, G4 and G5, who became ill 24 and 26 days after G1

House A The first four cases, A1 to A4, were boys who had attended the school A1 fell ill on 12th April and as his last attendance at school was on 20th March the minimal incubation period seemed to be 23 days The other three boys attended school until they fell ill on 17th 20th and 23rd April Eleven boys, A5 to A15, fell ill between 12 and 21 days after A1

had been infectious at this time,
he could have infected the eight boys just before or just after his isolation, and their incubation periods would then have been about 25 to 40 days A3 and A4 were also isolated with colds, from 27th March to 1st April and [illegible] to 24th March respectively, and might have infected other boys

In House A, the building-up of [illegible] early in the incubation period was school, probably because infectivity increased in several boys who had colds at that time and because exposure in the House was longer and closer than in the school

There were fifteen cases among the females within 27 days, from 1st to

27th May All the patients except one were confined to House A where their dining-rooms, day-rooms, dormitories, lavatories and washing accommodation were separate from those for the boys There was contact between the males and females in the yard where they exercised or sat on warm dry days The one patient with contacts outside the house was a girl who attended the school and fell ill on 8th May Some cases may have been infected by this girl during her incubation period or by the earlier cases among the males or by females infected from these sources The inmates of this house were low grade defectives and their filthy habits may have contributed to the spread of infection at a time when it rarely occurs among more cleanly persons Two nurses who attended the women and two other women fell ill 27, 32, 25 and 27 days after the first case and were presumably infected by the early cases

House N There were eight cases in schoolchildren and seven in children who did not go to school Two nurses also developed the disease They and all the children who did not go to school can be accounted for by contact spread from cases among schoolchildren with a 20- to 40-day incubation period Several children had colds in March and early April

Though there are gaps and uncertainties in the story, it is possible to trace case to case infection in many instances on the basis of a 20 to 40-day incubation period The more explosive outbursts may be explained on the same basis if it is assumed that cases may be infectious early in the incubation period and that concomitant upper respiratory infections coupled with dirty habits greatly increased the infectivity at every stage

Attack rates by sex age and House The attack rates on males and females of all ages were 52 9 and 10 2 per cent respectively This difference was partly accounted for by the larger proportion of females in the older less susceptible age groups (Table 22) In the 5 to 14 age groups the attack rates on males and females were similar The attack rate in 10-year age groups decreased from 54 1 per cent in the 5 to 14 group to 0 7 per cent in those aged 35 or over

TABLE 22

Age and Sex Distribution of Cases

Age	*Males*		*Females*		*Both sexes*		*Attack rates (per cent)*		
	at risk	*cases*	*at risk*	*cases*	*at risk*	*cases*	*males*	*females*	*both sexes*
5–14	25	14	23	12	48	26	56 0	52 2	54 1
15–24	22	13	185	31	207	44	59 1	16 8	21 9
25–34	4	0	167	9	171	9	0 0	5 4	5 3
35+	0	–	147	1	147	1	—	0 7	0 7
Total	*51*	*27*	*522*	*53*	*573*	*80*	*(52 9)*	*(10 2)*	*(13 9)*

The attack rates in Houses (Table 23) varied considerably, but after correction for the age distribution of the inmates (Table 24) it was clear that only those for Houses A and M differed from the expected rates House A with a high rate contained the lowest grade patients and House M with a low rate contained the highest-grade patients so that the explanation seemed to lie in the increased opportunities of spread of infection among the low-grade patients

TABLE 23

Attack Rates by Houses and Sexes

House	Category	Cases in males	Cases in females	Cases in both sexes	Attack rates (per cent) males	Attack rates (per cent) females	Attack rates (per cent) both sexes	Nurses at risk	Nurses cases
N	Children	4	11	15	44 5	52 3	50 0	6	2
G	Mixed grades	2	4	6	50 0	31 8	35 3	3	0
A	Lowest grade	21	15	36	55 2	19 5	31 3	20	2
B	Intermediate grade	–	10	10	–	8 7	8 7	11	1
M	High grade	–	13	13	–	4 4	4 4	47	0
	Total	27	53	80	(52 9)	(10 2)	(13 9)	87	5

TABLE 24

*Age Distribution in the Houses Observed and Expected Cases**

Age	Attack rate in Institution	House N At	House N Cases		House G At	House G Cases		House A At	House A Cases		House B At	House B Cases		House M At	House M Cases	
5—1																
15—2																
25—3																
35+																
Total	(13 9)	30	15	12 5	17	6	3 9	115	36	19 1	115	10	12 1	296	13	30 5

* Expected cases in each age group are calculated on the rates of column 2 except the total which is the sum of expected cases in the four age groups

HOMOLOGOUS SERUM HEPATITIS IN THE SAME INSTITUTION

By courtesy of Dr S A Propert, Colchester, and of Dr J R Hutchinson of the Ministry of Health records of the cases of homologous serum hepatitis in the Institution in 1937 were made available The epidemic of infective hepatitis described above, in conjunction with these records, provides some data on the relationships of homologous serum hepatitis and infective hepatitis

On 1st June 1937 seven children aged 5 to 11 were inoculated with 4 5 ml of convalescent measles serum All seven developed jaundice between 71 and 83 days later, from 10th and 22nd August all were seriously ill ... and three children died On 14th November, 3 ... in the inoculated children, two other ... had not received measles serum, but ... who developed jaundice after inoculation Both these children had severe attacks, one was delirious for 2 days, the other had convulsions muscular twitchings and opisthotonos for 4 days, but both recovered Dr Propert suggested that the two November cases were due to infection with the agent which produced the hepatitis in the inoculated children, but others held that these were cases of infective

hepatitis and that the disease was severe because the patients were mental defectives However, the experience of infective hepatitis in mental defectives recorded here, and experience in other Institutions in Region 4, have shown that infective hepatitis is not often severe in mental defectives If the first opinion is accepted the incubation period for naturally-transmitted homologous serum hepatitis was about 3 months as following parenteral inoculation Four other cases possibly infected in a similar fashion have been reported (McFarlan and Chesney, 1944, Bradley, 1946)

Three of the children who developed jaundice in 1937 after inoculation with measles serum were in the Institution in 1944 One was in House B and two were in House G Only one, an inmate of House G, developed hepatitis in the 1944 epidemic The two patients who developed jaundice in November 1937 apparently as a result of contact with the homologous serum cases were in Houses A and B in 1944 The one in House A developed hepatitis and as has been mentioned, after recovering from it died of bronchopneumonia and heart failure Thus two of the patients who were jaundiced in 1937 had not been immunized by their attacks, and one of them at least suffered then from homologous serum hepatitis There is other evidence both epidemiological (McFarlan and Chesney, 1944, Gauld, 1946) and experimental (MacCallum, see p 137) of the absence of cross immunity between the two diseases

Discussion

METHOD OF TRANSMISSION OF INFECTION

Contact A great bulk of evidence supports the view that infective hepatitis is spread by contact from case to case In cases studied in Region 4 there was a history of contact with a previous case between 20 and 40 days before the onset of illness in 18 9 per cent. of sporadic cases in Cambridge, and in 46 7 per cent of cases in village epidemics (Table 25) When contacts with cases whose onset preceded that of the case in question by less than 20

TABLE 25

Previous Contacts of Sporadic Cases in Cambridge and of Cases in Village Epidemics

Contact case	*Place of contact*	*Numbers*		*Percentage*	
		Sporadic cases in Cambridge	*Village epidemic cases*	*Sporadic cases in Cambridge*	*Village epidemic cases*
Onset 20—40 days previously	Home	5	14	18 9	46 7
	School	6	22		
	Elsewhere	2	6		
Onset less than 20 days previously	Home	5	3	8 7	13 3
	School	1	9		
No known contact		50	36	72 4	40·0
	Total	*69*	*90*	*100*	*100*

days were included, the percentages became 27 6 in sporadic cases and 60 0 in epidemics There is justification for including these cases in estimating the importance of contact because the probability is that both cases were infected by contact with the same source or that one was infectious early in the incubation period and the second was in fact due to contact with the first (p 36) In some rural epidemics the percentage of cases with known contact is higher than 60 per cent (Pickles, 1939, McFarlan 1941) In poliomyelitis the percentage of cases with a history of contact with a previous case varies similarly from about 25 to 80 per cent

Carriers of infective hepatitis An explanation put forward to explain the spread of poliomyelitis is that there are many carriers of the virus, some being healthy carriers or having symptomless infections others having an "illness of infection" or an abortive attack (Conybeare, 1946) From a careful study of village epidemics of infective hepatitis Pickles (1939) concluded *that carriers are not concerned in the spread of infection but that* careful search will always reveal a missed case Newman (1942) concluded from similar studies that carriers did transmit infection In Region 4 no instances of definite spread by carriers were observed although in many cases with no history of direct contact an effort was made to establish indirect contact through another member of the family However, some of the epidemiological findings strongly suggest that carriers existed Carriers seemed to be the most probable source of infection of the many sporadic cases and the origin of many epidemics *The first case in some village epidemics* appeared to have been infected while visiting a town after the epidemic there had ceased, which suggested that carriers were present in the town The immunity of older persons is probably due to the subclinical infections in early life for which Pollock (1945a) obtained some biochemical evidence Thus there are several lines of evidence pointing to the existence of healthy carriers Convalescent carriers presumably exist for Newman (1944) observed an institutional outbreak which seemed to originate from a patient who had a relapse 2 months after a first attack The evidence pointing to a period of infectivity early in the incubation period (p 36) suggests that precocious carriers should also be taken into account

Relative importance of pharyngeal secretions and faeces Epidemiological evidence suggests that infective hepatitis may be transmitted by pharyngeal secretions Casual contact sometimes apparently suffices to transmit infection as, for instance, in the case of an ambulance driver whose only known contact with a case of infective hepatitis was 30 days before her illness began when she sat for some minutes beside a patient in the waiting room of a hospital Newman (1942) records similar instances The winter incidence of the disease is compatible with a droplet spread In epidemics the capricious nature of spread is often noted For example, in a family a child A may sleep and play with child B, and rarely meet an older child C but if A develops hepatitis C may succumb a month later and B the month after that The [illegible] and in some towns is

[illegible]

of infective hepatitis in 1943-45

There are on the other hand findings compatible with a spread from faeces

In some outbreaks infection is spread not diffusely but only to close contacts Pickles (1939) found that close contact was usually necessary for the transmission of infection and that successive cases often occurred in children who slept in the same bed or frequently played together High attack rates were found in the Mediterranean area and in some villages in Region 4 where sanitary arrangements were poor and facilities for personal hygiene very primitive Human transmission experiments leave no doubt that faeces contain the infective agent

On the evidence, therefore, spread seems to occur from either the pharynx or the faeces In either instance contaminated fingers may play a part and any increase in closeness of contact or decrease in opportunities for personal hygiene may facilitate the spread of infection

WATERBORNE EPIDEMICS

Fraser (1931) described an outbreak of jaundice which followed 3 to 5 weeks after a waterborne outbreak of gastro-enteritis due to a *Salmonella* organism Neefe and Stokes (1945) described an epidemic of infective hepatitis which was explosive and had a high attack rate and in which infection was probably transmitted to many of the cases by well water heavily polluted with the faeces of cases Olin (1947) described a waterborne epidemic in guests at a hotel in Sweden Gauld (1946) showed that well-water was the probable source of infection of cases in some units of the American army in Italy The presence of the infective agent in water has been demonstrated by human transmission experiments, and the virus has been shown to be relatively resistant to chlorination (Neefe, Stokes, Baty and Reinhold, 1945) Waterborne outbreaks can therefore be expected, and Hallgren (1942 and 1943) maintains that they are common in Sweden His evidence is not, however, conclusive and the verdict of most investigators is that waterborne epidemics of infective hepatitis are rare

MILKBORNE EPIDEMICS

In an explosive epidemic of jaundice affecting about 150 schoolchildren in three day schools in Scotland ("J M," 1943), the infection appeared to be milkborne because the disease was confined to children who drank milk supplied to the schools by one dairy There was however no obvious source of contamination of the milk Selander (1939) quotes Wickstrom to the effect that spread by milk is unlikely because he had seen many cases in farm and dairy hands and no excessive incidence in the consumers of milk handled by them In Region 4 no evidence was obtained that milk was the vehicle of infection, and in two epidemics in day schools the attack rates were similar in those children who drank school milk and those who did not (Table 26) Milkborne epidemics of infective hepatitis appear therefore to be rare

TABLE 26

Attack Rates in Children who Did or Did Not Drink School Milk

Place	*Children who drank milk*			*Children who did not drink milk*		
	Total	*Affected*	*Attack rate (per cent)*	*Total*	*Affected*	*Attack rate (per cent)*
Bungay	475	97	20 2	291	57	19 6
Bishop s Stortford	700	74	10 6	369	27	8·0

CONTROL OF EPIDEMICS

the

occ

action is clear In most epidemics spread occurs by contact, either by the respiratory or the intestinal route or both This double mode of spread, coupled with the lengthy incubation period, the relatively low attack rate in contacts and the straggling nature of the epidemic, make it difficult to apply measures of control It may however be useful to discuss some of the measures which can be adopted

Early diagnosis Efforts should be made to diagnose cases in the pre icteric stage, and suspects should be treated as cases until the diagnosis is clear

Isolation of cases and suspects The rarity of the spread of infection from jaundiced cases in hospital to their attendants or to other patients suggests that no very stringent precautions are necessary in the established case In view of the extended series of cases in nurses which sometimes follows the introduction of infection among the nursing staff it is wise to insist on barrier nursing of cases with proper disposal of faeces during the first fortnight of illness Similar precautions should be taken with suspects, for a week from the onset of symptoms or until some other diagnosis is made During an epidemic at a school it is wise to exclude for a week all children showing suspicious symptoms, because not infrequently children stay away from school for a day with vomiting and malaise, returning during the infectious pre icteric stage only to develop jaundice a few days later Children who develop the disease can be allowed to return to school a fortnight after the onset of symptoms if they are then well

of epidemic it is impracticable to impose quarantine in an institution but after a more explosive outbreak quarantine for 6 weeks or so may be advisable In all outbreaks the most practicable way of dealing with contacts is to isolate them promptly if suspicious symptoms appear

Immunizatic ... with γ globulin might be an ... outbreak It is doubtful, how ... usual protracted epidemic

defaecation and before meals, and every possible ... that these facilities are used

References

J J, ii 268

BRESH D S (1944) *Trans R Soc trop Med Hyg* 38, 35
BURNET F M (1940) *Med J Aust* 1 325
CAMERON J D S (1943) *Quart J Med* 12 139
COLLINS, S D (1924) *Publ Hlth Rep Wash* 39 1559
CONYBEARE E T (1946) *Mon Bull Min Hlth Lond* 5 15
CULLINAN E R (1939) *Proc R Soc Med* 32 933
DIBLE J H and MCMICHAEL J (1943) *Brit J vener Dis* 19 107
EDWARDS, L. R L (1943) *Brit med J* 1 474
FINDLAY G M and MARTIN N H (1943) *Lancet* 1 678
———, ———, and MITCHELL J B (1944) *Lancet* ii 301
FORD J C (1943) *Lancet* 1 675
——— (1944) *Proc R Soc Med* 37 176
FOSTER M and GASKELL, J F (1916) *Cerebrospinal Fever* Cambridge University Press
FOX J P MANSO, C. PENNA, H A and PARA M (1942) *Amer J Hyg* 36 68
FRASER R (1931) *Canad Publ Hlth J* 22 396
GAULD R L (1946) *Amer J Hyg* 43 248
GIBSON C (1914) *Publ Hlth Lond* 27 181
GREENWOOD M (1939) *Epidemics and Crowd Diseases* Williams and Northgate, London
HALLGREN R (1942) *Acta med scand* Suppl. 140
——— (1943) *Acta med scand* 115 52
HAVENS W P PAUL, J R and VAN ROOYEN C E (1945) *Lancet* i 202
J M " (1943) *Mon Bull Dept Hlth Scot* 2 64
KIRK R (1945) *Lancet* 1 80
LISNEY A A (1944) *Proc R Soc Med* 37 165
MACCALLUM F O and BRADLEY W H (1944) *Lancet* ii 228
MCFARLAN A M (1941) *Publ Hlth Lond* 55 56
——— (1945a) *Quart J Med* 55 125
——— (1945b) *Lancet* 1 592
——— and CHESNEY G (1944) *Lancet* 1 816
MEDICAL OFFICERS OF THE MINISTRY OF HEALTH (1943) *Lancet* 1 83
NEEFE J R and STOKES, J (1945) *J Amer med Ass* 128 1062
NEEFE J R STOKES, J BATY J B and REINHOLD J G (1945) *J Amer med Ass* 128 1080
NEWMAN J L (1942) *Brit med J* 1 61
——— (1944) *Proc R Soc Med* 37 171
OLIN G (1947) *Acta med scand* Suppl 196 381
OLIPHANT J W (1944a) *Bull N Y Acad Med* 20 429
——— (1944b) *Publ Hlth Rep Wash* 59 1614
PICKLES, W N (1939) *Epidemiology in Country Practice* Wright Bristol
POLLOCK M R (1945a) *Brit med J* ii 598
——— (1945b) *Lancet* ii, 626
PROPERT S A (1938) *Brit med J* ii 677
RUSSELL, W T (1943) *Spec Rep Ser Med Res Coun Lond* No 247
SAWYER W A MEYER K F EATON M D BAUER J H PUTNAM P and SCHWENTKER F F (1944) *Amer J Hyg* 40 35
SCHOOL EPIDEMICS COMMITTEE (1938) *Spec Rep Ser Med Res Coun Lond* No 227
SELANDER P (1939) *Acta paediatr Stockh* 23 Suppl 4
SMITH R E (1935) *Practitioner* 135 283
SPOONER E T C (1944) *Proc R Soc Med* 37 171
SPURLING N SHONE J and VAUGHAN J (1946) *Brit med J*., 2, 409
VON BORMANN F BADER R E DEINES H and UNHOLTZ, K (1943) *Hepatitis epidemica in Deutschland* 1937-8 *Beitr z Hyg u Epidem* 1 Leipzig
WILLARD J H (1944) *Nav med. Bull Wash* 42 1085
WILLIAMS, H (1923) *J Amer med Ass* 80 532
WILLCOX W H (1919) *Brit med J* 1 671

II CLINICAL OBSERVATIONS

by

CLIFFORD WILSON D M F R C P

DURING the period of these observations (November 1943—September 1945) some 2 000 cases of infective hepatitis among Service personnel were notified in Civil Defence Region 4 an area embracing Cambridge and the country lying between the river Thames and the Wash Three hundred of these cases were admitted under members of the Jaundice Team for observation and therapeutic trials The vast majority were young male subjects but otherwise the cases were unselected *

Symptoms and signs were recorded at least twice weekly The urine was examined daily for bile and other abnormal constituents by trained nursing staff and the results were frequently checked against independent examinations in the laboratory a rough estimate of the degree of biluria was obtained using the foam test † Patients were weighed twice weekly No specific dietary restrictions were made except that greasy foods were with hel[illegible]

by [illegible]

the [illegible]

but sample analysis of the food actually consumed gave the following [illegible] protein [illegible] n of the [illegible] calorific value 3,500 No routine treatment was given except that ape[illegible]nts were administered when necessary

In general complete rest in bed was enforced until the urine had been bile free (using the foam test) for 7 days as a rule the serum bilirubin had fallen below 2 mg per 100 ml at this time Patients were then allowed up for toilet for a further 3 to 4 days and were permitted increasing activity during the following 10 to 14 days before discharge to a convalescent depot After 3 to 4 weeks graded training at the depot a final examination was made The period of rest in bed was extended after severe attacks or relapses and for patients who showed persistent liver enlargement and tenderness or a delayed fall in serum bilirubin

Signs and Symptoms

When this investigation began the relationship of catarrhal jaundice to infective hepatitis and the status of homologous serum and arseno therapy hepatitis had not been clarified Detailed attention was therefore paid to the clinical features of these conditions Similarly the identity of the epidemic form of infective hepatitis occurring in the Mediterranean area with the sporadic type prevalent in this country had not been established The present series contains 73 cases arising in Service units which had returned to this country from the Mediterranean theatre during the 1943-44 epidemic and these have been compared with cases arising during the same period in home-based units There were no consistent differences in the

* Cases were referred to the Jaundice Team as soon as infective hepatitis was suspected or diagnosed and were admitted to hospital if accommodation was available

† *Foam Test* The urine is shaken in a test tube if bile pigment is present the foam takes on a yellowish tinge *Small amounts of bile can be recognized by* comparison with a normal urine

clinical picture or laboratory findings between the two groups, and hence no separation is made in the general analysis which follows

The age distribution of the first 200 cases of the series is shown in Table 27, and the percentage incidence of their symptoms and signs is recorded in Table 28

TABLE 27

Age Distribution

Age group	*No of cases*	*Percentage*
17–19	22	11 0
20–24	63	31 5
25–29	54	27 0
30–34	34	17 0
35–39	24	12 0
Over 39	3	1 5
	Total 200	

TABLE 28

Percentage Incidence of Symptoms and Signs

Symptom and sign	*Infective hepatitis* (200 *cases*) *Percentage*	*Arsenotherapy hepatitis* (34 *cases*) *Percentage*
Anorexia	93 5	76 5
Nausea	78 0	85 0
Headache	58 5	44 0
Lassitude	58 5	44 0
Vomiting	50 5	35 5
Shivering	50 0	29 5
Constipation	46 0	41·0
Abdominal discomfort	41 0	41·0
Abdominal pain	36 5	11 8
Backache	35 5	26 5
Pains in limbs	21 5	44 2
Retro-orbital pain	15 0	5 8
Upper respiratory symptoms	17 0	8 8
Skin irritation	13 0	11 8
Diarrhoea	11 0	5 8
General aches	10 5	2 9
Dizziness	9 0	2 9
Skin rashes	10 0	8 8
Pain on eye movement	7 0	2 9
Jaundice	*100 0*	*100 0*
Liver enlargement	83·0	73 4
Liver tenderness	88 0	73 4
Bradycardia	73 0	72 8*
Fever	43 5	45 5*
Splenic enlargement	16 5	22 8*
Pale stools	90 0	86 0*
Dark urine	88 0	94 2

* This figure is based on 22 cases admitted to the Jaundice Unit

In general, infective hepatitis is a disease of abrupt onset, it begins with a combination of febrile and digestive symptoms among which the most prominent are lassitude, anorexia, shivering, frontal headache, muscle and joint pains, abdominal pain or discomfort, nausea and vomiting After a variable interval, usually of 1 to 10 days, the pre icteric stage is followed by the appearance of dark urine and jaundice At this point there is, in most instances, a rapid improvement in symptoms The liver is commonly enlarged and tender and the spleen may be felt Unless the attack is particularly severe or a relapse occurs, all signs and symptoms have disappeared in 3 to 5 weeks

The general features of infective hepatitis occurring in the various theatres of war have been adequately described elsewhere There are certain aspects which have only been generally appreciated as a result of war-time experience and which deserve particular emphasis The most significant of these is the frequency, at the onset, of symptoms suggesting an acute infective process Fever or febrile symptoms, such as shivering and sweating, were present in three quarters of the present series Rigors were uncommon but pyrexia up to 103° F (39° C) was not unusual Frontal headache, retro orbital pain, physical and mental lassitude, generalized aches in muscles and joints, particularly affecting the limbs and back, were characteristic presenting symptoms There was then not infrequently a temporary improvement, with subsidence of fever, followed by a return of symptoms, particularly digestive ones, and by a secondary rise in temperature about the time when jaundice first became apparent

Attempts have been made in the past to differentiate between jaundice with severe constitutional symptoms, which was attributed to infective hepatitis, and jaundice with few symptoms which was diagnosed as "catarrhal jaundice" The present series does not support any such demarcation All grades of severity were encountered both in sporadic and epidemic cases In a large epidemic many severe cases may be seen in a short space of time and these may give the false impression that the disease is more severe than, or clinically different from, that observed in sporadic cases There appear to be no clinical grounds for retaining the concept of "catarrhal jaundice" as a disease entirely distinct from infective hepatitis

Certain rare general symptoms are of special interest Headache is occasionally associated with neck rigidity simulating meningitis Blurring of vision and pain on eye movement may accompany retro orbital pain Skin irritation and urticaria may be presenting symptoms Other skin rashes erythematous or macular, and small crops of petechiae have been very occasionally observed

Digestive symptoms are rarely absent in infective hepatitis They may be associated with the febrile symptoms at the onset or may appear during or even after the pre-icteric stage Loss of appetite is the commonest of all symptoms, it is particularly prominent with greasy foods Non greasy articles of diet containing fat, including eggs, milk, cheese and even butter, are well tolerated Abdominal pain is usually epigastric, but may be central, hypogastric or localized in the right upper or lower quadrants It may be of such severity as to suggest perforated ulcer or it may simulate the pain of acute appendicitis *Abdominal discomfort* is usually described as a heavy, cramp like or blown up feeling in the epigastrium and may be associated with flatulence or severe heartburn Diarrhoea is not a common

symptom It appeared in 11 per cent of the present series In a few cases it was attributed to an independent gastro enteritis but in general the onset appeared to coincide with the appearance of acholic stools at the end of the pre-icteric stage

Of particular interest is the association of upper respiratory tract infection which has been described in some outbreaks of infective hepatitis It is not uncommon for the patient to complain of a "cold" or "influenzal symptoms" at the onset of an attack of infective hepatitis, but on close enquiry the symptoms involved are usually headache, shivering or pains in the limbs which can be recognized as initial symptoms of the disease Upper respiratory symptoms, coryza, cough and sore throat, are indeed rare at the onset A burning sensation or discomfort in the throat is occasionally due to acid eructation or is associated with retrosternal or epigastric discomfort In the present series a history of upper respiratory symptoms before or during the attack was obtained in 34 patients (17 per cent) Twenty-eight of these complained of coryzal symptoms, sneezing running cold, cold in the head and cough 2 had nasal catarrh with obstruction, 2 complained of cough with muco purulent sputum and 2 of sore throat, the latter symptom was also recorded in 2 of the patients with coryza The duration of upper respiratory symptoms was commonly 1 to 3 days, but 7 patients complained of a persistent cold for 3 to 4 weeks The interval between the respiratory infection and the onset of infective hepatitis was very variable, ranging from a few days to over 4 weeks When the occurrence of respiratory symptoms was related to the monthly distribution of admissions it was found that 31 of the 34 cases occurred between October 1943 and April 1944 giving an incidence over this period of 25 5 per cent The remaining 3 occurred during the months May to September of 1943 and 1944 giving an incidence of 3 8 per cent The high incidence from October to April was partly due to the admission over this period of a large number of men on their return from the Mediterranean area In this group the incidence of respiratory infection as a prodromal symptom of infective hepatitis was 40 per cent, it appeared however that the great majority of these troops suffered from colds on board ship or on arrival in this country In short, the relationship of upper respiratory infection to infective hepatitis was inconstant in this series, coryzal symptoms showed no uniform time relationship to the onset of the disease, and their incidence was almost entirely confined to the winter months and was highest in a group of men returned to the United Kingdom at a time when the common cold was prevalent In a few instances upper respiratory symptoms coincided with the onset of an attack of infective hepatitis and it is possible that in these and others, the preceding infection may by lowering of resistance, have predisposed the patient to the attack In general, however, it seems that respiratory symptoms were coincidental and not an integral part of the disease

Liver enlargement and tenderness occurred in 83 per cent and 88 per cent of cases respectively, and as they may be present in the pre icteric stage these signs are of early diagnostic value There is often marked muscular resistance in the right upper quadrant so that it is important to feel for the liver edge external to the rectus muscle Splenomegaly was present in 16 5 per cent of cases, the enlargement being two or three fingerbreadths below the costal margin, and usually associated with tenderness In a few cases

splenomegaly persisted after all other signs and symptoms disappeared. The inconstancy of palpable splenomegaly in infective hepatitis, its early occurrence, the associated splenic tenderness and its apparent independence of the severity of liver damage all suggest that involvement of the spleen is a separate manifestation of the disease and not a result of portal congestion. The histological findings in patients dying in the early stages indicate an acute inflammatory process rather than simple congestion and the clinical observations support this view. The fact that splenomegaly so often persists, with or without signs of residual liver damage, is of interest in its possible relationship to certain cases of "splenic anaemia" in which chronic enlargement of the spleen is found in the presence or absence of cirrhosis of the liver.

Enlargement of superficial lymph glands was looked for in this series but was not found with sufficient constancy to be regarded as an integral feature of the infection.

Course of the Disease

The clinical course of infective hepatitis is remarkably constant and free from complications. After the onset, which is usually sudden, general and digestive symptoms commonly persist for a short period before the appearance of jaundice. At the end of this pre-icteric stage symptoms improve rapidly and by the time jaundice is fully developed the patient complains of little except a subnormal appetite, lassitude and some degree of epigastric discomfort. Icterus usually increases during the following week and then gradually diminishes over a period of 1 to 2 weeks. In many cases minor digestive symptoms, occasional headache, and undue fatigue after exertion persist for several weeks after the disappearance of jaundice, but for most a return to normal activities is possible 4 to 5 weeks after the onset. In this series the pre-icteric stage varied from 0 to 24 days and was usually 1 to 8 days in duration (Table 29). Occasionally, before the onset of jaundice, there is a temporary improvement followed by a return or aggravation of symptoms. This remission during the pre-icteric stage has been frequently described but it was observed in only 15 cases in this series. In most of these there was an apparent dissociation of general and digestive symptoms. Some of the general symptoms, headache, lassitude, shivering, backache and pains in the limbs, were present at the onset in 13 cases, whilst in the remaining 2 urticaria was the sole initial symptom. As a rule during the remission the patient felt comparatively fit and returned

TABLE 29

Interval between Onset of Symptoms and Appearance of Jaundice

Interval before jaundice (days)	*No. of cases*	*Percentage*
0– 3	57	28.5
4– 6	58	29
7– 9	41	20.5
10–12	19	9.5
13–15	11	5.5
Over 15	14	7
	Total 200	

to duty After a further 1 to 8 days symptoms returned, shivering or fever recurred in half the cases, lassitude, headache and backache usually returned, and in one of the men who had presented with urticaria the rash reappeared The most outstanding symptoms after the remission were complete loss of appetite, nausea and vomiting

During the icteric stage jaundice was commonly observed to increase in depth for a period which in this series varied between 3 and 10 days Parallel with the development of jaundice there was an increase in biluria and the stools remained pale Symptoms steadily improved during this time in all but the most severe cases After a variable time during which the skin colour remained unchanged, jaundice began to fade, at first rapidly and then more slowly The rapid phase was associated with a rapid fall of the serum bilirubin, the slow

During the peric the other signs dimini hen jaundice appeared became normal after a mean total duration of anorexia of 11 8 days Lassitude had a similar but slightly longer duration Liver enlargement and liver tenderness underwent a parallel diminution The mean interval from the onset of symptoms to disappearance of liver tenderness was 28 0 days It was often observed that in individual cases liver tenderness was more persistent than jaundice In 125 cases in which the duration of hepatomegaly (measured from the onset of symptoms) could be estimated, the liver remained enlarged for as long as 2 weeks in 38 cases, from 2 to 4 weeks in 40 cases, from 4 to 8 weeks in 40 cases and from 8 to 13 weeks in 7 cases As stated previously, patients were not discharged from hospital until jaundice had disappeared, the urine had become bile-free and the serum bilirubin was below 2 mg per 100 ml Even when these conditions were satisfied the patient was kept in bed if the liver remained enlarged and tender Exactly half the total number of cases remained in hospital for one month or less, of the remainder, 80 were discharged after 4 to 8 weeks, while 7 patients were in hospital for 3 months or more

Of the first 200 cases of the series 101 were referred back without selection for follow-up observations after one month's graduated training at a convalescent depot Particular enquiry was made for digestive symptoms and undue fatigue after marching and physical training, a general examination was made, the urine was tested for urobilin and bilirubin, and the serum bilirubin and bromsulphalein excretion were estimated if there was any suggestion of incomplete recovery Symptoms and signs were absent and the biochemical tests were negative in 54 instances in the remainder residual manifestations included excessive fatigue on exertion, indigestion, abdominal pain, headache, backache, liver enlargement and tenderness, and enlargement of the spleen Enlargement of the liver or spleen without symptoms was found in 14 cases, liver enlargement or tenderness with residual symptoms occurred in 25 and symptoms alone in 8 so that one third of the patients in this unselected group who had left hospital feeling quite well returned after one month's training with some subjective disorder Four patients returned with a definite relapse, apart from these no abnormality was discovered by the biochemical tests at our disposal

tic

st

grouping was also found in a small series of cases examined at intervals up to one year after the initial attack The first group consisted of patients who complained of persistent or periodic digestive symptoms, abdominal pain, especially in the right upper quadrant, and fatigue, on examination, enlargement of the liver with or without splenomegaly was found Some of the attacks, which were commonly precipitated by strenuous exercise or indulgence in alcohol, were accompanied by a return of jaundice to the sclerotics, and frank relapses occasionally occurred Patients in the second group continued to be free from symptoms but the liver, and less frequently the spleen, remained enlarged Whether in these groups the liver lesion is resolving or progressing will be determined only by a longer period of observation Thirdly there was a minority of patients with no demonstrable liver enlargement but with residual symptoms, particularly fatigue, indigestion and headache, which tended to be constant rather than periodic, and which were out of proportion to the severity of the initial attack Whilst it was impossible to exclude the possibility of residual liver damage, the clinical picture in these instances was more suggestive of a neurosis

RELAPSES AND RECURRENCES

From a long term study of infective hepatitis among civilians Pickles (1939) concluded that one attack confers an immunity comparable with that of measles Among military personnel, reports of second attacks are not uncommon, but it is possible that many of these are relapses following shortly after a first attack with incomplete recovery Out of 272 Service cases examined by the writer there was a history of a previous attack of jaundice in 26 instances The interval between the two attacks was 8 to 22 years in five, 7 months to 3 years in ten and less than 3 months in eleven cases It seems likely that the five patients with an interval of 8 years or more suffered from re infection This presumption is less certain in the second group with intervals of 7 months to 3 years There was apparently complete recovery from the first attack and freedom from symptoms in the interval If men in the first two groups are presumed to have suffered from second attacks the recurrence rate would be 5 8 per cent Seven of these cases had intervals of 3 years or more and the recurrence rate was then 2 6 per cent In the eleven cases with less than 3 months interval it appeared certain that the second attack was a relapse following incomplete recovery Three were ambulant during the first attack and three returned to duty before jaundice had disappeared This gives the impression that inadequate rest during an attack of infective hepatitis predisposes to a relapse While this may be so, similar relapses are as frequently seen before patients have been allowed out of bed

Relapses were observed during their period of hospital treatment in 23 out of the 200 patients in the series (11 5 per cent) All cases are included in which there was a return or aggravation of symptoms with either an increase in jaundice or biochemical evidence of deterioration of liver function Relapses were of varying severity, some exceeding the initial attack in intensity and duration, others producing only a slight return of biluria and increase in serum bilirubin, with temporary loss of appetite or malaise lasting not more than 1 or 2 days The intervals between the onset of the initial attack and the relapse showed a uniform scatter from 14 to 60 days Three patients had two relapses In 13 instances the initial jaundice had disappeared

before symptoms returned The most common relapse symptoms were anorexia, lassitude, nausea, vomiting and headache The temperature which had previously been normal was raised in 4 cases Jaundice returned or increased in 17 patients and was associated with an increase or recurrence of biluria and rise in serum bilirubin in 15 who were investigated at the time Following severe relapses the liver enlarged and became more tender In 3 patients the spleen, which had not previously been felt, became palpable Biochemical investigations show that hippuric acid excretion may fall steeply with the return of symptoms, whilst a granulopenia, similar to that which is seen at the onset, may occur at the same time An interesting feature is the absence of any pre-icteric interval during the early relapse The return of symptoms, jaundice and biluria, and the rise in serum bilirubin appear together The explanation may be that the pre icteric interval usually observed at the onset is partly determined by the functional reserve power of the liver, and that this is considerably reduced during the early stage of recovery When relapses have occurred late in convalescence after all signs of liver damage have disappeared, the interval between onset of symptoms and appearance of jaundice has again been observed

Although it has been suggested that both alcohol and strenuous exercise may precipitate late relapses of infective hepatitis, neither of these factors could have been operative in the majority of relapses described above In these patients there was no apparent precipitating factor and it is concluded that reactivation of the disease may occur spontaneously Complicating infections arising during the recovery period appear to have no effect on the course of the disease Febrile illnesses due to tonsillitis or pharyngitis (8 cases), pneumonia (1), otitis externa (1) and pyelitis (1) produced no return of biluria or other signs of relapse

Comparison of Epidemic Cases from the Mediterranean Area and Sporadic Cases among Home-based Troops

The return to the United Kingdom of certain units from the Mediterranean area provided an opportunity for making a comparison between the epidemic form of infective hepatitis and the sporadic type occurring in home units This revealed a remarkable resemblance in symptomatology, severity and duration of the disease in the two groups

SYMPTOMATOLOGY

The percentage incidence of symptoms is given in Table 30 It will be seen that the order of frequency of symptoms is almost identical in the two groups whilst the actual percentage incidence shows a remarkable parallelism The epidemic in units from overseas continued for several months in this country and there was a possibility that cases arising later than one month (the average incubation period) after disembarkation might have been infected outside their units Cases arising later than one month after arrival in England have therefore been excluded from column 3 in the Table To make the comparison more exact cases in home-based units admitted before the return of C.M F units have also been eliminated, the 58 cases in column 4 were studied over the same period as the C M F cases In these more selected groups the above relationships are confirmed

Two features require special comment, diarrhoea and upper respiratory symptoms (including coryza and sore throat) which were at times associated

with infective hepatitis during the epidemics of 1942 and 1943 in the Mediterranean area Our figures reveal no significant difference in the incidence of diarrhoea between the groups Upper respiratory symptoms showed considerable variation They were commonest (40 per cent) in the corrected groups of C M F and this is explained by the high incidence of colds in these troops on their return to England Cases of infective hepatitis arising after the first month in England had a much lower incidence of upper respiratory symptoms (8 per cent), since the common cold epidemic had then subsided In the Home Forces group there were no "preceding respiratory symptoms" before 1st November 1943, and 17 per cent after this date Thus in both groups (C M F and H F) upper respiratory symptoms were often absent and when present may, as previously suggested, have been coincidental and not an integral part of the disease

TABLE 30

Percentage Incidence of Symptoms of Infective Hepatitis in Cases from Mediterranean Area (C M F) and Home Forces (H F)

Symptom	Total		Corrected*	
	C M F (73 cases)	H F (79 cases)	C M F (37 cases)	H F (58 cases)
Anorexia	85	92	89	90
Dark urine	86	86	84	88
Nausea	73	70	84	66
Headache	58	57	49	52
Vomiting	53	50	62	40
Lassitude	48	55	35	57
Shivering	44	47	43	40
Epigastric discomfort	40	43	40	42
Epigastric pain	31	35	30	31
Backache	27	34	24	29
Pains in limbs	15	27	8	22
Generalized aches	4	12	0	14
Irritation of skin (pre icteric)	3	12	3	11
Diarrhoea	10	9	11	9
Preceding upper respiratory symptoms	25	13	40	17

* *Corrected groups*
C M F—excludes cases arising later than 1 month after return to England
H F—excludes cases arising before return of C M F to England

SEVERITY AND DURATION OF THE DISEASE

It is difficult to find a satisfactory single criterion for the severity of infective hepatitis, since in individual cases the various features of the disease show such an inconstant relationship to one another A combination of clinical and biochemical data was therefore used In Table 31 the percentage distribution of the different degrees of jaundice and liver enlargement is shown for the four groups the percentage of cases showing persistent liver enlargement on discharge from hospital is given and the incidence of relapses is tabulated for each group The maximum observed serum bilirubin levels are compared in 57 C M F and 48 H F cases in Table 32

Criteria used to indicate the duration of the disease were (1) duration of anorexia, (2) interval between first symptom and disappearance of bile from the urine, (3) duration of jaundice, (4) interval between onset and disappearance of liver tenderness, (5) interval between first symptom and return of serum bilirubin to 2 mg per 100 ml, (6) period in hospital

TABLE 31

Severity of Infective Hepatitis in Cases from Mediterranean Area (C M F) and Home Forces (H F)

Criterion		Total		Corrected*	
		C M F (73 cases)	H F (79 cases)	C M F (38 cases)	H F (58 cases)
Maximum degree of jaundice (Per cent distribution)	+	53	45	58	54
	++	35	34	28	29
	+++	12	21	14	17
Maximum degree of liver enlargement (Per cent distribution)	0	22	22	20	20
	+	48	36	44	43
	++	17	32	17	26
	+++	13	10	19	11
Persistent liver enlargement (Per cent)		15	16·5	14	17
Splenomegaly (Per cent)		16·5	19·0	22·5	22·5
Relapses (Per cent)		9·6	12·6	8·5	15·5

* *Corrected groups*
C M F—excludes cases arising later than 1 month after return to England
H F—excludes cases arising before return of C M F to England

TABLE 32

Duration of Infective Hepatitis in Cases from Mediterranean Area (C M F) and Home Forces (H F)

Criterion	Total					Corrected*				
	C M F		H F			C M F		H F		
	Mean	S E of Mean	Mean	S E of Mean	D/S E	Mean	S E of Mean	Mean	S E of Mean	D/S E
Duration of anorexia (days)	10·7	1·05	11·8	1·00	1·10	10·1	1·55	11·7	1·25	0·80
Duration of biliuria (days)	20·9	1·31	22·0	1·28	0·60	21·1	2·27	23·3	1·63	0·79
Duration of jaundice (days)	18·6	0·96	19·4	1·13	0·47	19·7	1·27	19·4	1·81	0·14
Duration of liver tenderness (days)	26·3	2·65	24·7	2·77	0·42	22·3	2·84	28·3	4·16	1·32
Period in hospital (days)	32·5	2·80	32·6	2·18	0·03	35·0	3·58	32·7	2·10	0·55
No of cases	57		48							
Mean maximum serum bilirubin (mg per 100 ml)	7·9		10·3		2·0	—		—		—
Mean interval from onset to 2 mg serum bilirubin level (days)	21·5		28·3		2·4	—		—		—

* *Corrected groups*
C M F—excludes cases arising later than 1 month after return to England
H F—excludes cases arising before return of C M F to England

A comparison of the criteria of severity shows a very similar percentage distribution of the degrees of jaundice and liver enlargement in both the total and "corrected" C M F and H F groups The incidence of persistent hepatic enlargement is also similar It might be expected that splenic enlargement would be commoner in troops who had been exposed to malaria, in fact this was not the case (Four C M F cases admitted to hospital with malaria and jaundice have been excluded) A close correspondence was also found for the criteria indicating duration of the disease Biochemical

tions reveal that this slight difference was due to a small number of patients in the Home Forces group with an unusually high and prolonged elevation of the serum bilirubin Haematological investigations were made on the same series of patients and revealed no significant difference in blood picture between the two groups (p 94)

We may conclude that a study of the symptomatology severity and duration of epidemic infective hepatitis in troops returned to this country from the Mediterranean area provides no reason for believing that the disease is a different entity from infective hepatitis occurring sporadically in Service units in England

Non-icteric Cases of Infective Hepatitis

Until a specific test for infective hepatitis is discovered the recognition of the disease in a patient with typical symptoms rests on clinical or bio chemical evidence of liver damage and in particular on the appearance of biluria and increase in serum bilirubin This series of cases presented a wide range of severity of liver involvement and in a few jaundice was so slight that the diagnosis might well have been missed under less favourable conditions In most cases the rise in serum bilirubin was sufficiently prolonged to produce severe jaundice, in occasional patients however a steep rise and fall in serum bilirubin was associated with only slight and transient jaundice It is probable that when the duration of hyperbili rubinaemia is short, jaundice either does not occur or is too slight to attract attention Other biochemical changes may be equally transient so that a patient may give a typical history of infective hepatitis and yet show neither jaundice nor biochemical evidence of liver involvement when he first seeks attention The total and differential white counts are of uncertain value since in this series both were normal n nearly half the cases and even if characteristi found not specific for the disease The diagnosis hepatiti be confidently made in the absence of ja bioch are positive, where neither clinical nor is obtained but typical occur c is a history of cont prev sis tive but cannot be m non Over

In gat ission

the it

of

Pati the

ranean

Medical Officers had had considerable experience in the early recognition of the disease Fifty such cases were admitted during the period of this investigation, in only three was the diagnosis of infective hepatitis subsequently made and jaundice developed in all of these The remainder were suffering from a wide range of conditions including malaria, glandular fever, lobar pneumonia, gastro-enteritis, ascariasis, dysentery, tonsillitis, influenza and diphtheria It is however possible that at the height of an epidemic (such as was prevalent in the Mediterranean theatre in 1942 and 1943) non-icteric cases occur and are instrumental in spreading the infection A further possibility is the occurrence of the infection with no involvement of the liver It is obvious that unless repeated observations are made from the onset, using sensitive liver function tests, slight and transient liver involvement may be overlooked The recognition of such cases, if they exist, and of carriers who exhibit neither symptoms nor jaundice must await the development of a specific test for the disease

Relation of Symptoms to Lesion

The clinical picture of infective hepatitis presents three main aspects which must be considered in assessing the severity of an attack and in discussing the relationship of symptoms to the underlying lesion (1) the onset symptoms, (2) the manifest effects of liver damage—jaundice, liver enlargement, biliuria and bilirubinaemia and (3) residual symptoms which may persist for weeks or months after the attack These phases of the disease correspond roughly with the pre icteric, icteric and post-icteric stages The use of the term "pre icteric stage" may erroneously suggest that the liver is unaffected until the end of this period In fact, investigation of pre-icteric cases indicates that liver damage occurs at or soon after the onset, and by the time icterus is manifest liver function is usually recovering The interval which is commonly observed between the onset of symptoms and the appearance of jaundice is due partly to the fact that the rise in serum bilirubin does not begin for several days and partly to the gradual nature of the rise in many instances The varying rate of rise explains why different levels of serum bilirubin are found when jaundice is first detected

The probability that liver damage occurs at the onset, and that it is frequently maximal in the early stages of the disease suggests a parallel with the symptomatology and raises the question of the relationship of symptoms to liver damage The nature of liver dysfunction is too obscure to provide direct proof but there is much presumptive evidence that the initial symptoms of infective hepatitis are not due to liver damage It is common experience that symptoms may be minimal in patients with severe, protracted jaundice and considerable hepatic enlargement Moreover the time relations of symptoms and jaundice are inconstant, in several of our cases symptoms appeared several days after jaundice was established During a relapse of [illegible]

disturbance In order to discover whether the production of digestive symptoms is related to the degree of liver enlargement, the percentage

there is no reason to believe that they represent a selected sample Individual cases gave the impression of a more severe degree of liver damage than was seen in infective hepatitis and this is confirmed by a comparison of the two groups The data presented in the last column of Table 33 show that in severity and duration the arsenotherapy cases most closely resembled the severest group of infective hepatitis cases, i e those in which the serum bilirubin exceeded 10 mg per 100 ml The incidence of relapses was dispro portionately high, 55 per cent of cases suffering a relapse or exacerbation during the recovery period This is significantly higher than the relapse rate for the most severe group of infective hepatitis cases In sixteen of the arseno-therapy cases the maximum serum bilirubin rose above 10 mg per 100 ml and in eleven it exceeded 15 mg per 100 ml so that the correlation between the clinical criteria of severity and serum bilirubin level is borne out by this group

Although the degree of liver damage was greater, the symptoms were in general no more severe than in infective hepatitis The percentage incidence of symptoms and signs for the two groups is shown for comparison in Table 28 (p 51), only one symptom, pain in the limbs, was notably commoner in arsenotherapy hepatitis The pain was referred to the muscles rather than the joints, and the arms and shoulders were most commonly affected, in a few patients muscle pains preceded the other symptoms of hepatitis by days or weeks A further apparent point of difference was the common persistence of certain symptoms after admission to hospital, particularly nausea, vomiting and abdominal pain or discomfort This is comparable with the behaviour of severe cases of infective hepatitis, but more pronounced, possibly owing to the greater frequency of exacerbation or relapses during the icteric stage of the disease In these relapses the manifestations of liver damage were more marked than in the similar relapses of infective hepatitis Recovery of liver function was slower in arsenotherapy hepatitis than in infective hepatitis so that there is a greater probability of relapses occurring while the liver is still damaged The degree of hepatic enlargement in arsenotherapy hepatitis was significantly greater than in the most severe grade of infective hepatitis, in six patients marked hepatic enlargement was still present on discharge from hospital after all other evidence of liver damage had disappeared The frequency of splenomegaly (22 8 per cent) was not significantly greater than in infective hepatitis

It was suggested above that the high attack rate of arsenotherapy hepatitis might be explained by the parenteral mode of transmission for similar high rates are usual in homologous serum hepatitis Reports of the latter, in particular those of yellow fever vaccine jaundice, do not suggest that liver damage is more extensive than in infective hepatitis It is therefore possible that the greater severity of arsenotherapy hepatitis is attributable to the additional hepatotoxic action of arsenic

HOMOLOGOUS SERUM HEPATITIS

During the course of the investigation considerable attention was paid to the possibility of hepatitis arising as a sequel of blood or plasma transfusion The ten cases encountered were admitted to four hospitals in the region during the 8 months from April to November 1944 Four were civilians who received reconstituted serum or plasma following abdominal operations, three were also given whole blood The same batch of plasma

was used in two instances, the others received plasma at the same hospital but the batch numbers could not be traced One patient died after jaundice developed, although the hepatic lesion was severe, other conditions found post mortem were sufficient to cause death The ages of these patients were 53, 58, 62 and 63, none gave a history of contact with infective hepatitis and the intervals between the transfusion and the development of symptoms of hepatitis were 63, 64, 90 and 103 days The remaining six were Service patients Two had received whole blood together with serum or plasma transfusions in this country and four had been given serum or plasma abroad, in one instance followed by whole blood The intervals between transfusion and the onset of hepatitis were 53, 62, 70, 76, 79 and 90 days Two of these patients died 5 and 6 days after jaundice appeared and the histological findings were typical of acute necrosis of the liver In none of the Service cases was any history of contact with a case of jaundice obtained

GLANDULAR FEVER

The occurrence of jaundice in glandular fever is now well recognized Martin (1941) found thirteen cases in the literature and since then others have been recorded Kilham and Steigman (1942) reported twenty cases of glandular fever, of which seven developed jaundice, liver biopsy in one of these revealed acute inflammatory changes resembling those of infective hepatitis

During the present study twelve patients with glandular fever were investigated, all showed the typical blood picture and a positive Paul Bunnell reaction Seven were jaundiced on admission and three were referred to hospital as possible pre icteric cases of infective hepatitis There was therefore, considerable selection in this group and no conclusions can be drawn as to the frequency of liver involvement in glandular fever The degree of liver involvement was less severe than is usually encountered in infective hepatitis, and jaundice, which eventually occurred in eight cases, was slight and of short duration The following characteristics were observed biluria in all twelve cases palpable liver in seven (five jaundiced), liver tenderness in nine (seven jaundiced) palpable spleen in six, with tenderness on palpation in three, tenderness under the left costal margin, without a palpable spleen in two

The occurrence of jaundice was apparently unrelated to any particular clinical aspect of glandular fever Four cases were of the anginose type (one developed jaundice), generalized enlargement of the superficial lymph glands was found in seven (four jaundiced) persistent fever occurred in two (one jaundiced) Three patients who were admitted with jaundice presented no clinical features suggesting glandular fever, but their disease was differentiated from infective hepatitis on the basis of the blood picture and was confirmed by the Paul-Bunnell reaction

In general, and whether jaundice appeared or not, the symptoms resembled those of infective hepatitis The pre icteric interval varied from 4 to 12 days, dark urine was noted in six of the eight patients who became jaundiced Anorexia was a symptom in twelve cases and occurred at the onset in nine, headache and shivering were each recorded in nine cases, [illegible]

irritating generalized papular erythema In the latter case there was a remission of symptoms in the prodromal stage similar to that observed in some cases of infective hepatitis, headache, lassitude and irritation occurred before the remission and anorexia, abdominal pain nausea and vomiting after it, associated with the appearance of dark urine

MALARIA

Haemolytic jaundice is a recognized feature of severe malarial infection In occasional patients jaundice of the non haemolytic type associated with biluria and acholic stools, is observed When both malaria and infective hepatitis are prevalent, as during the 1943 epidemic in the Mediterranean zone, it may be impossible to decide whether the jaundice is due to malaria alone or to combined infection This problem was encountered during the present investigation Many cases of malaria developed in troops returned from abroad, and amongst the patients referred to us as possible cases of infective hepatitis twenty were found to be suffering from benign or malignant tertian infection Jaundice was observed in seven of these cases and in four of them bilirubin was present in the urine and the serum bilirubin gave an abnormally high direct Van den Bergh percentage It was impossible to distinguish these cases from infective hepatitis by biochemical tests The [illegible]

of severe rigors at regular intervals was in marked contrast to the slight and transient shivering of infective hepatitis, headache was more often generalized in the malaria cases and pain below the left costal margin was not uncommon On examination liver tenderness was the rule but palpable enlargement was slight and was present in less than half the cases Splenomegaly was occasionally greater than in infective hepatitis enlargement to 3 to 6 fingersbreadths below the costal margin being found in five patients These distinctive features of malaria were not sufficiently constant for the clinical differentiation of malaria and infective hepatitis in all cases and consequently it was impossible in patients with non haemolytic jaundice to decide whether malaria alone or a combined infection with malaria and infective hepatitis was the cause of the liver disorder

Prognosis

The foregoing discussion indicates the main prognostic features of infective hepatitis Clinical and biochemical observations suggest that complete recovery is the rule, although slight residual symptoms and signs often persist for weeks or even months after jaundice has disappeared Persistent liver enlargement and tenderness are the commonest signs of incomplete recovery, and tend to be more frequent when exacerbations or relapses have occurred during the course of the attack The severity of the onset symptoms appears to bear no relation to the ultimate course of the disease There is no clear evidence on the effects of inadequate rest in the early stages of the disease although a number of patients we have seen during a relapse were ambulant during the initial attack In a series of cases of yellow fever vaccine jaundice Turner and his colleagues (1944) have described certain psychotic symptoms which were regularly followed by death from acute

necrosis of the liver Similar manifestations have been observed in a few fatal (civilian) cases of infective hepatitis encountered during the present investigation There was in these patients nothing to suggest an unusually severe attack until mental symptoms, particularly irritability, excitability and disorientation, appeared Coma rapidly supervened, with periods of violent excitement, and death followed in a few days All recent reports agree that acute necrosis of the liver is a rare sequel of infective hepatitis During the two-year period of this study more than 2,000 Service cases of infective hepatitis have been notified to us and, apart from the two instances in which plasma transfusion had previously been given, no deaths have been recorded

Differential Diagnosis

Difficulties in the diagnosis of infective hepatitis may arise before or after the development of jaundice During the pre-icteric stage the condition is commonly mistaken for other diseases which produce similar symptoms In the Middle East, sandfly fever and malaria presented the chief difficulties In the United Kingdom the combination of fever with headache, pain in the limbs and loss of appetite is most likely to lead to a provisional diagnosis of influenza Where digestive symptoms predominate gastro enteritis is frequently diagnosed The association of fever, severe headache and backache with granulopenia may suggest typhoid fever Meningitis is suspected in the occasional cases where severe headache is accompanied by stiffness of the neck muscles The early appearance of a macular erythema has led to the diagnosis of German measles Abdominal symptoms at the onset may be so severe as to suggest perforated ulcer or acute appendicitis and pain and tenderness in the right iliac fossa not infrequently lead to anxiety even after the appearance of jaundice A differential white count is of value in these circumstances since a granulopenia is usually present in the early stages of infective hepatitis whilst such a finding makes the diagnosis of acute appendicitis unlikely

Unless the disease is epidemic, recognition of infective hepatitis in the pre-icteric stage is most difficult except in the rare instances where a history of contact is obtained The only other symptom of diagnostic value is the appearance of dark urine, for the majority of male patients notice this several days before jaundice appears It may be possible to establish the diagnosis earlier than this by using sensitive laboratory tests (Pollock, 1945) The most valuable of these are Hunter's test for biluria and the bromsulphalein excretion test In a doubtful case the differential white count may be of assistance

After jaundice has appeared the common difficulty is to distinguish between infective hepatitis and extrahepatic obstruction when no certain cause for the latter can be found This difficulty can often be resolved by careful enquiry into the mode of onset, for the pre-icteric symptoms of infective hepatitis, though common in other infectious diseases, are not likely to precede an attack of simple obstructive jaundice The age factor is of limited value in detecting the disease, we have admitted to hospital within a short time of each other a case of carcinoma of the pancreas in a man of 36 and a patient with infective hepatitis at the age of 63

Jaundice associated with persistent or irregular fever, particularly if this exceeds 100°F (38°C), is rare in infective hepatitis and suggests cholangitis or cholecystitis, it should also bring to mind the possibility of glandular

fever or Weil's disease In the absence of significant enlargement of the superficial lymph glands the differential diagnosis between glandular fever with jaundice and infective hepatitis depends on the blood picture and the heterophil antibody reaction If pyogenic infection can be excluded, a leucocytosis or shift to the left in the polymorphonuclear cell count of a patient with jaundice is an indication for the specific tests for Weil's disease A leucocytosis is however, common in the late stages of acute hepatic necrosis

Treatment

The time-honoured treatment of infective hepatitis is a low fat diet The rationale of this treatment is uncertain, but there are several reasons why reduction of fat in the diet may appear desirable The most compelling reason is the repugnance frequently felt by the patient for fatty, or more correctly, greasy foods , furthermore, restriction of fats might be expected to contribute towards physiological rest for the damaged liver although in fact the liver is equally concerned in the metabolism of other food constituents , there is also the consideration that absorption of fat is deficient in the absence of bile from the intestine, although this taken alone might be an argument for increasing rather than reducing the amount of fat ingested Animal experiments have demonstrated that a high fat diet may predispose to liver damage but the proportion of fat in such diets has been excessive when compared with the content of the normal human diet The main objection to the low fat diet is the difficulty in restricting fat alone It is not easy to maintain an adequate caloric intake when fats are excluded , the removal of cream, butter, eggs, cheese and meat fat also eliminates the fat soluble vitamins although over a short period of time this may be unimportant It also causes a considerable reduction in essential protein which may, however, be countered by adding skimmed milk The increased prevalence during the second world war of infective hepatitis, homologous serum hepatitis, arsenotherapy hepatitis and toxic hepatitis due to industrial poisons stimulated interest in the relationship of dietary factors to liver damage The relative importance of various dietary factors in producing experimental liver damage is still uncertain but recent work suggests that a low protein intake and in particular a deficiency of the sulphur-containing amino-acids is more important in the production of acute liver damage than a high fat diet Daft, Sebrell and Lillie (1942) showed that in rats cysteine or methionine prevented the

... deficient diets Himsworth and Glynn

... idependent

... monstrated

that in protein depleted dogs necrosis of the liver due to chloroform poisoning could be prevented by methionine Goodell, Hanson and Hawkins (1944) found similar protective action against the hepatotoxic action of "mapharsen" The conditions of these latter experiments were highly artificial but the liver-

... that its therapeutic

... issessing the

... is from the

... aximal soon

after the onset and most patients are well on the ... when they are admitted to hospital so that it is impossible to obtain a large series of cases in which treatment is started early enough to prevent liver damage In

the majority, the disease is mild and recovery is rapid whatever the treatment. Finally, even if cases of moderate severity are accessible in the early stages, the large individual variation in the criteria used to assess severity and rate of recovery would necessitate both a large series and an unequivocal therapeutic effect to provide a convincing answer The results of amino acid therapy in liver disease should therefore be examined most critically It must be remembered that the dramatic effects in animal experiments have been the result of substitution therapy and no comparable protein deficiency has been demonstrated in patients who contract infective hepatitis The metabolic functions of the sulphur-containing amino-acids are obscure and their present use in man is empirically based on a hypothetical deficiency or inactivation of these substances in liver disease Their practical usefulness, if any, in infective hepatitis would lie in the promotion of more rapid recovery, in a reduction of the relapse rate and the prevention of the most serious sequelae of the disease—acute liver atrophy and subacute necrosis

Therapeutic trials were carried out during this investigation to determine the effect on the course of infective hepatitis of methionine, cysteine and of varying the fat content of the diet Detailed results have been published elsewhere (Wilson, Pollock and Harris, 1945, 1946) Oral administration of methionine and cysteine seemed to accelerate recovery, but the differences between treated and control patients were slight and their statistical significance was not fully established It is improbable that these substances will prove of practical therapeutic value, but further trials with them might help to elucidate some of the problems of liver metabolism Comparison of the recovery rate on diets of different fat content indicated that a low fat diet had no discoverable advantage and that generous diets high in fat content, were acceptable to the great majority of patients

References

[illegible]

Ann intern Med, 20 193

WILSON C, POLLOCK M R, and HARRIS A. D (1945) *Brit med J*, i 399

WILSON, C, POLLOCK, M R and HARRIS A D (1946) *Lancet*, i 881

III. BIOCHEMICAL OBSERVATIONS

by

M R POLLOCK M B

THE nature of the biochemical studies in this investigation has largely been determined by the immediate requirements of the Jaundice Team In particular, these requirements have been

(a) a means for the detection of cases of infective hepatitis as early as possible in the course of the disease, i e in the absence of jaundice,
(b) a method for the assessment of liver function, in order to determine and compare the efficacy of specific treatments

A method of detecting cases *in the absence of jaundice would have especial* value in studies of the epidemiology of the disease and it would facilitate the collection of infective samples of blood and excreta for transmission experiments *The biochemical studies have therefore developed mainly along* two lines

(a) an investigation of the stage of the disease before the appearance of jaundice (pre-icteric stage), and of cases who never actually develop jaundice (sub icteric cases),
(b) examination of liver function in patients during the acute stage of the disease and in early convalescence while in hospital

For (a) the need was mainly for simple tests which could be performed in large numbers, since the patients and contacts to be studied were numerous and had usually to be investigated during an epidemic in their homes or in institutions For (b) the requirement was a reliable test of liver function which could be frequently repeated on the same patient and which could be performed satisfactorily in the presence of jaundice

Our experience has not included many seriously ill patients since these were rare, and the biochemical results refer mainly to what can fairly be called the average case Investigations have consisted of repeated estimations, with only a few tests of simple technique, on a large number of mild or moderate cases of the disease Until very recently most of the biochemical work on infective hepatitis has not been systematic, but rather in the nature of comparative studies of the more severe cases, or investigations on the differential diagnosis of jaundice and the relative value of various liver function tests The approach chosen was designed to help in the elucidation of the relationship of jaundice to the pathology of infective hepatitis Why, in fact is there retention of bilirubin in the blood and what is happening to the liver before jaundice appears?

Details of the Biochemical Tests

Test and technique	*Normal mean value*	*Adopted upper limit of normal*
(1) Serum bilirubin Malloy and Evelyn (1937)	0 47 mg per 100 ml	1 3 mg per 100 ml
(2) Direct quantitative Van den Bergh reaction Modification of Malloy and Evelyn s method in which the intensity of the colour developing 1 minute after addition of the diazo reagent to the serum is expressed as a percentage of the total final colour developed after further addition of alcohol	5	10

Test and technique	*Normal mean value*	*Adopted upper limit of normal*
(3) Urine bilirubin Hunter's test	negative	trace
Iodine ring test	negative	negative
(Hunter's test is approximately twenty times more sensitive than the iodine test)		
(4) Urine urobilin and urobilinogen Spectroscopic test	+	+++ (equivalent to a positive Schlesinger test at a urine dilution of 1 in 5)
(5) Bromsulphalein excretion test 5 mg/Kg body weight dose Estimation of percentage dye retention in serum 30 minutes after intravenous injection	trace (<5)	7 5
(6) Intravenous hippuric acid test Quick (1939) 1 77 g dose of sodium benzoate given intravenously Estimation of hippuric acid synthesized and excreted in the first hour following the injection	not estimated	0 80 g sodium benzoate in 1 hr

Full details of the above tests with criticisms, normal standards and some values obtained in other diseases have already been published (Pollock, 1945a, c) The tests were carefully chosen after an exhaustive search in the literature and some practical experience during the first months of work Hunter's test and the bromsulphalein excretion test were used during the pre-icteric stage and convalescence only, the hippuric acid test (H A) was only practicable in hospital and thus rarely performed in the pre-icteric stage It was, unfortunately, not often possible to obtain admission of cases to hospital before the appearance of jaundice and our investigations on the pre-icteric stage were usually done on different patients from those available for tests during jaundice and convalescence

Pre-icteric and Sub-icteric Cases

A complete account of our findings during the pre-icteric stage of 53 cases of infective hepatitis has already been published (Pollock, 1945a) Results of the bromsulphalein excretion test (97 per cent positive), Hunter's test for bilirubinuria (86 5 per cent positive) and the 1 min direct Van den Bergh percentage (90 per cent positive) suggested that liver function was deranged in most cases a considerable time (up to 10 days) before the appearance of jaundice and often very shortly after the onset of symptoms Moreover, these tests were found to be of considerable assistance in the early diagnosis of the disease It is true, of course, that the tests are also liable to be positive in certain other diseases malaria and infective mononucleosis, for example, quite often gave rather similar results, and were also liable to be confused clinically There seems now little doubt that the biochemical similarities between the three diseases are due, at least in part, to the fact that hepatitis may occur in all Kilham and Steigman (1942) have described an acute hepatitis in material obtained by liver biopsy from a case of glandular fever, and Cohn and Lidman (1946) have produced biochemical evidence of liver damage in the same disease There are several reports of abnormal liver function in cases of malaria The bromsulphalein excretion test is, moreover, liable to show abnormal retention in diseases where there is no other evidence of liver dysfunction Here the problem appears to be one of adopting a satis-

factory standard of blood retention, if this is fixed too low, the test is not of much value for diagnosis

Further findings in the pre-icteric stage have been a serum bilirubin and urine urobilin-plus-urobilinogen within normal limits until very shortly (2 to 3 days) before the appearance of jaundice This is, no doubt, partly due to their very wide normal variations, but it also seems that the liver's reserve powers of excretion for the two substances are not usually exhausted until the disease has continued for some time Although both tests have been commonly used in the past for diagnosis of infective hepatitis, it does not now appear that they are comparatively of such value as a sensitive test for bilirubinuria In addition, excess urinary urobilin was found to occur almost as frequently in a miscellaneous group of "non-hepatic" diseases Very similar results have been obtained by Gellis and Stokes (1945) using the methylene blue test for bilirubinuria, and by Barker, Capps and Allen (1945a) who also found, amongst other evidence of liver damage in the pre-icteric stage, bilirubinuria and positive bromsulphalein excretion tests, and a normal serum bilirubin until shortly before jaundice, they also mention the uselessness of tests for urine urobilin in early diagnosis It does not seem that Hallgren's (1942) claim to have demonstrated liver damage in the incubation period up to 3 weeks before jaundice, purely on the basis of raised serum bilirubin values, is justified

The problem of sub-icteric cases of infective hepatitis is closely similar to that of the pre-icteric stage There appears to be a fairly general belief in their existence, but disagreement as to their frequency In one institutional epidemic (p 38), out of 85 cases with typical symptoms we found 7 (Pollock, 1945a) which gave abnormal liver function tests but never developed definite jaundice Altogether 13 such cases have been investigated In general, the picture was like that of the pre-icteric stage of an ordinary attack without the ensuing jaundice, and was characterized by bilirubinuria (often very considerable), an abnormal dire[illegible]
tion test, and a serum [illegible] s
were reported by Barke[illegible]
Up till recently, estimations as to their frequency lay between 1 6 per cent (Williams, 1923) and 9 per cent (Bates, 1936)—figures of the same order as ours, but Gowen (1945) has claimed, on the dubious evidence of raised values of the serum icteric index, that cases without jaundice, including "asymptomatic subclinical cases," may be 8 times as frequent as those with jaundice Even Barker *et al* (1945a) state that sub-icteric cases are 2½ times as common as those with jaundice These large discrepancies are probably partly due to lack of sufficiently sensitive liver function tests on the one hand, and to too great a readiness to diagnose sub-icteric cases on the other The criterion we have used for their diagnosis has been the combined presence of (a) typical symptoms, (b) history of contact with a known (jaundiced) case and (c) biochemical evidence of liver damage Although this criterion is somewhat [illegible] of their frequency, the
[illegible] r cent reten-
[illegible] used for the
[illegible]
[illegible] stage further
[illegible] an outbreak
[illegible] hout jaundice

but in the absence of symptoms Hunter's test for bilirubinuria was used on 17 child contacts thrice weekly for 10 weeks, positive results were obtained in 3 children in the absence of significant symptoms, and, in one further case a positive result was obtained 2 days before the onset of symptoms Control tests on 32 other children of the same age in two other nurseries were uniformly negative over a period of 4 weeks

Difficulty in the diagnosis of these subclinical and sub icteric cases must remain one of the possible factors in the spread of the disease That they exist must be accepted as proved, the only uncertainty is their frequency

Later Stages of the Disease

The progress of the disease after the appearance of jaundice has been described by other workers (Cullinan, 1938, Cameron, 1943, Barker *et al* 1945a, etc) and there is no need to present a full account here Our general findings have supported most other work The serum bilirubin rises rapidly to a peak and falls, at first rapidly and then much more slowly, and gradually returns to normal The degree and extent of bilirubinuria approximately follow the hyperbilirubinaemia, but usually a few days in advance, i e bilirubinuria first appears before the rise in serum bilirubin begins and has usually disappeared before the serum bilirubin reaches normal limits in convalescence (see Table 34) Excess urinary urobilin and urobilinogen occurs at the same time as the beginning of the rise in serum bilirubin, and again during the period of rapid fall in serum bilirubin, the intervening period being characterized by absence of urine urobilin and the presence of pale-coloured stools Cases vary widely in duration and severity, and relapses may occur at any time In our experience these relapses may be anything from a slight clinical setback with little or no biochemical change (or *vice versa*) to a complete second attack often more severe than the first They appear to show just the same range of severity as primary attacks, and rises in serum bilirubin may or may not occur, but an increase in, or return of, bilirubinuria is almost invariable Similar findings have been reported by Gellis and Stokes (1945)

TABLE 34

Relation between Serum Bilirubin and Disappearance of Bilirubin from Urine in Convalescence

Test	*Maximum recorded serum bilirubin (mg per 100 ml)*	*No of cases*	*Mean level of serum bilirubin* (with standard errors in brackets) when test for bilirubinuria became negative (mg per 100 ml)*	*D/S E cases above and below 8 0 mg per 100 ml*
Iodine	Above 8·0	37	5 5 (± 0 57)	3 80
	Below 8 0	25	3 1 (± 0 27)	
Hunter's	Above 8·0	25	2 0 (± 0·164)	2 77
	Below 8 0	30	1 4 (± 0 044)	

* Bilirubin values were estimated from the curves by interpolation (where necessary between the nearest recorded results at a time half way between the last positive and the first of 3 negative urine tests

It is clear that the serum bilirubin level is not a satisfactory measure of liver function, since considerable liver damage may be present without any *significant rise in* the serum bilirubin, and in any case the bilirubin excretory function should be assessed on the rate of bilirubin excretion from the liver, which is more closely related to the rate of rise or fall of the serum level than to the serum level itself We thus might expect maximum liver dysfunction to occur before the peak serum bilirubin level is reached Use was therefore made of the intravenous hippuric acid test (Pollock, 1945c) which was performed at least three times on each of 81 patients, on admission to hospital, a week later and shortly before discharge The results, which are summarized in Table 35, show clearly that there was significant improvement in hippuric acid synthesis in all groups during the first 6 days after admission to hospital In the more *severe cases admitted relatively early* in the disease (Subgroup (1) in Table 35) there is a suggestion that this improvement coincided with a continued rise in serum bilirubin, the mean value increasing from 9 5 to 12 45 mg per 100 ml during the same period, but although this undoubtedly occurred in individual cases, the increase in serum bilirubin was not great enough to be considered statistically significant The hippuric acid synthesis, in fact, bore some relation to the stage of the disease and the severity of the attack, but was only indirectly related to the *serum bilirubin level The conclusion is that maximum damage to liver* function probably occurs at the end of the pre icteric stage or very shortly after the onset of jaundice

Recovery of Liver Function in Convalescence

It is a matter of some importance to assess the extent and persistence of residual liver damage following an attack of infective hepatitis Unfortunately, findings on the subject have been to some extent contradictory H A tests on 81 patients shortly before discharge from hospital (on an average about 4 weeks after admission) showed complete return of the majority to normal function (Pollock, 1945c, these results are summarized in the last section of Table 35) In ac [illegible] test was used on 28 patients within 4 [illegible] per 100 ml, only 6 gave reten[illegible] 2 of these 6 were in the early stages of a definite relapse On the whole, the return of the bromsulphalein test to normal was remarkably rapid A further series of 14 were tested at times varying from 36 to 170 days after the serum bilirubin level had reached 2 0 mg per 100 ml, and only one patient had a *retention* of more than 7 5 per cent Hunter's test and serum bilirubin estimations on a larger number of cases on return from convalescence (usually from 6 to 8 weeks after admission) failed to show any abnormality Bilirubinuria tests in 57 were all *negative Serum bilirubin estimations* on 63 gave a mean value of 0 6 mg per 100 ml (very slightly above the normal mean of 0 47 mg per 100 ml), 4 were above 1 0 mg per 100 ml and 2 above 1 3 mg per 100 ml, but a subdivision of the group into those with *slight* residual symptoms or tender livers (30) and those with no complaints (33) revealed *no difference* between the mean serum bilirubin values (0 60 mg per 100 ml in both subgroups)

Other workers have, however, found indications of persistent liver damage following the disease Both Rennie (1943) and Gordon (1943) using the oral H A test have produced evidence suggesting a much slower return to

TABLE 35

Mean Values of Serum Bilirubin and Hippuric Acid Synthesis during the Course of the Disease in 81 Cases of Infective Hepatitis

Serum bilirubin group	No. of cases	On admission: H.A.*	On admission: Serum bilirubin (mg. per 100 ml.)	First week after admission: H.A.*	First week after admission: Serum bilirubin (mg. per 100 ml.)	First week after admission: Days after admission	Comparison of results on admission and after one week: H.A.*: Difference	H.A.*: D/S.E.	Serum bilirubin: Difference	Serum bilirubin: D/S.E.	Before discharge: H.A.*	Before discharge: Serum bilirubin (mg. per 100 ml.)	Before discharge: Days after admission
A. *Rising on admission*													
(1) Max. above 10 mg. per 100 ml.	14	0·527(±0·0376)	9·50(±1·04)	0·653(±0·0504)	12·45(±1·125)	6·0(±0·405)	+0·126	2·02	+2·95	1·94	1·048(±0·0528)	1·61	38·8
(2) Max. below 10 mg. per 100 ml.	23	0·689(±0·0478)	4·64(±0·474)	0·850(±0·0523)	5·22(±0·486)	5·5(±0·351)	+0·161	2·19	+0·58	0·86	1·060(±0·0306)	1·10	27·2
B. *Falling on admission*													
(3) Admission value above 5 mg. per 100 ml.	17	0·678(±0·054)	10·25(±0·436)	0·993(±0·0583)	3·86(±0·622)	6·7(±0·404)	+0·315	3·96	−6·39	2·55	1·155(±0·0398)	1·05	28·3
(4) Admission value below 5 mg. per 100 ml.	27	0·683(±0·042)	4·61(±0·341)	1·008(±0·0314)	1·83(±0·213)	6·3(±0·290)	+0·325	6·06	−2·78	6·92	1·081(±0·0317)	0·75	27·0

* H.A. — Hippuric acid synthesis in terms of sodium benzoate

normal Soffer and Paulson (1934) and Kornberg (1942), using the highly sensitive bilirubin excretion test, claim to have demonstrated liver damage in 80 per cent and 63 per cent respectively of old cases of "catarrhal jaundice" up to 18 years after the attack Noth and Loew (1943) claim to have detected liver damage in convalescent cases of "acute hepatitis" by means of the colloidal gold test *after the bromsulphalein test had become normal* Recently, Barker, Capps and Allen (1945b), basing their diagnosis on both clinical and laboratory findings, have reported the presence of a "*chronic hepatitis*" *in 76 out of 431* patients 4 months or more after the onset of an attack of acute infective hepatitis

It must be admitted that the question of liver damage following infective hepatitis is still undecided Residual dysfunction is probably small in the majority but may come to have some significance in the presence of other disease It still remains a serious possibility that a proportion of cases never fully recover and may later develop a subacute or chronic cirrhosis with ultimately fatal outcome

The Direct Van den Bergh Reaction

Ever since Van den Bergh made his famous discovery of the difference between *the so-called direct and indirect reacting bilirubins* of the blood, [illegible] become a useful [illegible] mism was due to [illegible] r or not there are [illegible] a exhibiting res pectively a direct and indirect reaction is a difference in the rate at which the bilirubin present reacts with the diazo reagent to produce the red azobilirubin pigment The nature of the reaction must therefore, if it is to mean *anything*, be expressed as a rate and not as a quantity Recently the true significance has come to be realized and the direct reaction is now more commonly being expressed as the proportion between the intensity of colour developed *in a standard time in* the absence of alcohol (or other catalyst) and the total colour developed after addition of alcohol (Lepehne, 1942, Ducci and Watson, 1945) This practice is altogether more satisfactory than attempts to measure quantities of "*direct bilirubin*" *or to express the reaction semi-quantitatively* in numbers of plus signs [illegible] me for measuring the direct reaction most commonly chosen is [illegible] id [illegible] " to give the best results The "1 min V [illegible] is, then, an arbitrary measure of the initial speed of the reaction between [illegible] the diazo reagent normal standards can be established in [illegible]

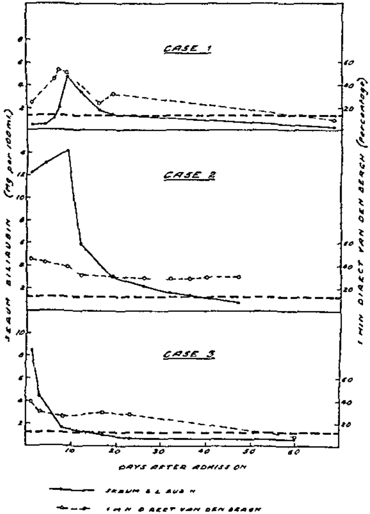
reaction [illegible] In our experience there is relatively little correlation

tration

speed of the direct V d B reaction and the serum bilirubin level, with the exception that, in infective hepatitis, low V d B values (< 20) are very rarely found in sera having a raised total bilirubin content

The transformation of indirect reacting to direct reacting bilirubin is widely held to be an exclusive function of the liver It might, therefore, be expected that the direct V d B reaction *could* act, in the absence of extra

hepatic obstruction as a liver function test It might be argued, for instance that an abnormal direct V d B reaction is an indication of the presence in the blood of the abnormal "direct" bilirubin and that this means the liver is failing to excrete it as fast as it should While this is a possible explanation the problem is clearly a good deal more complicated and it is preferable at present to withhold a definite opinion



[illegible]

Results obtained, using the 1 min V d B percentage during the pre icteric stage have already been summarized (Pollock 1945a) Values of 25

to 40 per cent were common The main interest is that a qualitative change of *bilirubin* from the indirect to the direct type preceded a quantitative increase in total bilirubin

Serial estimations of both the V d B reaction and total serum bilirubin have been made from once to twice a week throughout the disease in over 100 patients It is not possible to analyse these results satisfactorily as a group, but 3 cases are illustrated in Fig 15 Values of from 40 to 55 per cent for the 1 min direct V d B percentage were usual for the early part of the icteric stage and there was a gradual decrease as the patient improved, although fluctuation was considerable The peak V d B percentage occurred most frequently before the maximum serum bilirubin out of 32 cases admitted with rising serum bilirubin, the peak V d B value occurred before the peak bilirubin value in 21, was coincident in 9, and occurred afterwards in only 2 During convalescence, abnormal V d B values persisted for a surprisingly long time, usually many days or weeks after the total serum bilirubin had reached normal limits Table 36 summarizes a comparison of the two estimations in the period after the bilirubin level had reached 2 0 mg per 100 ml It can be seen that after fifteen days 31 out of 38 patients had a total serum bilirubin within normal limits while only 2 had a normal V d B reaction Even after thirty days, 22 out of 52 had abnormal V d B values

Bilirubinuria

One of the most interesting facts that have emerged on the biochemical side of this investigation has been the relative independence of bilirubinuria and hyperbilirubinaemia It was at one time thought that there was a renal threshold for bilirubin at a blood level of about 2 0 mg per 100 ml However, Rabinowitch (1932) showed that bilirubin could appear in the urine while the serum bilirubin level was normal, and our findings during the pre icteric stage have clearly demonstrated that no apparent renal threshold was operative It has been suggested that this may be due to renal damage but there is no real evidence in support of this As a rule renal damage has only been found in severe cases of infective hepatitis, and urea clearance tests performed on 12 unselected cases on admission to hospital (Pollock, 1945c) all gave normal values

Although no quantitative estimation of total urinary excretion of bilirubin was possible, it was fairly clear that the curve of the rise and fall of bilirubinuria is chronologically in advance of the serum bilirubin curve Another way of expressing the same fact would be to say that there is much greater bilirubinuria at any given serum bilirubin level when the serum bilirubin is rising than when it is falling This "discrepancy" is even more marked if bilirubinuria is compared with jaundice intensity A further point of [illegible] bin level at

[illegible] maximum serum bilirubin level above 8 0 mg. per 100 ml , [illegible] test first became permanently negative at a mean serum bilirubin of 2 0 mg per 100 ml , in those with peak values below 8 0 mg per 100 ml the corresponding serum bilirubin figure was 1 4 mg per 100 ml In one severely jaundiced case, for instance, the bilirubinuria ceased at a serum bilirubin level of 3 4 mg per 100 ml , as compared to 0 5 mg per 100 ml in a

TABLE 36

Direct Van den Bergh and Total Serum Bilirubin Results After Attainment of Serum Bilirubin Level of 2·0 mg per 100 ml

Time after attainment of 2·0 mg serum bilirubin level (days)	Total serum bilirubin (mg per 100 ml)	No of patients with a 1 min Van den Bergh percentage of				Total no of patients	Percentage with normal serum bilirubin	Percentage with normal direct Van den Bergh
		0—10	11—20	21—30	31 and over			
0—15	2·0—1·35	0	3	12	9	71	66	0
	1·3—0·75	0	5	23	14			
	0·7—0	0	2	3	0			
16—30	2·0—1·35	0	1	6	0	38	81	5
	1·3—0·75	1	6	9	4			
	0·7—0	1	4	5	1			
31 and over	2·0—1·35	1	0	0	0	52	96	57
	1·3—0·75	5	3	0	0			
	0·7—0	24	14	5	0			

Note 1 Double lines indicate the upper limits of normal

2 Not more than one result per patient is recorded in each time group (When more than one estimation has been made on a patient during one time-period only the first result is recorded)

patient with a mild attack who barely developed any jaundice at all In other words, the renal excretion of bilirubin depends upon other factors besides the serum bilirubin concentration

Bensley (1933) states that bilirubinuria appeared to be associated with a positive direct V d B reaction, and With (1943) maintains that there is no renal threshold for "direct" bilirubin At first sight our results, using the 1 min V d B percentage values, appear to confirm this point of view During the major part of the disease bilirubinuria and the direct V d B reaction appeared to be closely related They were both positive during the pre-icteric stage—in fact, no patient at this period was found to have an abnormal V d B reaction without concomitant bilirubinuria—and they both probably reached their maximum values together, before the peak serum bilirubin was attained In convalescence, however, there was a divergence, and abnormal V d B reactions were repeatedly found in the absence of bilirubinuria For instance, no patient was discharged from hospital before three consecutively negative Hunter's tests for bilirubinuria had been obtained, while about 50 per cent were found to have left hospital with abnormal V d B results Even if the persistent abnormal V d B in convalescence is not a reflection of a damaged liver but simply due to the continued drainage of abnormal bilirubin from the tissues back into the blood, it would be expected to be associated with some detectable bilirubinuria if With's hypothesis is correct The solution to the problem of bilirubinuria and the direct V d B reaction remains largely unsolved This does not, however, detract from the use of sensitive tests for bilirubinuria, such as Hunter's test or the methylene blue test, for the detection of liver damage in the absence of jaundice, their simple technique has made them of considerable value both as hospital discharge clearance tests for the early detection of relapses and in the investigation of contacts or suspicious cases in epidemics

Discussion and Conclusions

Conclusions on liver function are notoriously dangerous Apart from the fact that so little is known about the normal physiology of the liver, suspicions are continually and often justifiably being entertained that even the more reputable liver function tests may be influenced by factors apart from the liver itself The main question is how much can biochemical investigations contribute to the understanding of the pathology of infective hepatitis? Objections have on occasion been expressed against every one of the tests used in this investigation the H A test has been accused of being a kidney function as well as a liver function test, the bromsulphalein excretion test has been criticized as being "unphysiological" and liable to be influenced by disturbances of the whole reticulo endothelial system, the serum bilirubin level and bilirubinuria are, of course, affected by any interference with the outflow of bile into the intestine, and the direct V d B reaction is still so little understood that any interpretation is naturally viewed somewhat sceptically It would be possible to multiply such objections almost indefinitely and in a sense they must be accepted as reasonable Nevertheless, if certain safeguards are taken and the limits of the various tests fully appreciated, their use may be justified In the present study of infective hepatitis we have therefore attempted to build up a tentative and very incomplete picture of the probable biochemical course of the disease, and to draw certain practical conclusions

Any such study is based on the firm foundation of the liver biopsy findings of Roholm and Iversen (1939) and Dible, McMichael and Sherlock (1943) The existence of a true hepatitis with early and widespread disorganization of liver structure and cell integrity is now established Recovery appears in most cases to be rapid and complete As far as it goes, the biochemistry supports the histopathology Early abnormal biochemical findings suggest that damage to the liver occurs shortly after the onset of symptoms The main pathological changes are in the liver and they appear to occur early, some time before the appearance of jaundice or gross biluria The second conclusion is that maximum liver damage occurs before the peak serum bilirubin value is reached, and, from the point of view of the clinician, early in the disease—probably about the time of onset of visible jaundice or very shortly afterwards Here the objection may be raised that liver damage is difficult to define, and that different liver functions may be dissociated from one another Maximum liver dysfunction in relation to H A synthesis, for instance, may well occur at a different time from maximum derangement of the liver's bilirubin excretory power Dible *et al* (1943) have recorded the presence of so-called "bile thrombi" in the biliary capillaries and suggestions have been made that bilirubin retention may be due to some micromechanical intrahepatic obstruction rather than to a metabolic defect in the excretory ability of the hepatic parenchyma One of the more remarkable facts about infective hepatitis is that the appearance of jaundice so often coincides with clinical improvement This apparent association is probably a reflection of the fact that jaundice usually only becomes manifest towards the end of the acute phase of the disease which is itself not commonly protracted But it would appear at first sight to afford support for the idea of dissociated liver dysfunction and to fit in with the suggestion that hyperbilirubinaemia is due to internal hepatic obstruction following on as a later stage of liver damage which began as a purely metabolic disorder It is possible that this is so Our only contention is that it is not, on the biochemical evidence, necessary to assume any such dissociation of function In the first place, the presence of bilirubinuria in the pre-icteric stage suggests a defect of the liver's bilirubin excretory function early in the disease In so far as there is evidence that bromsulphalein is excreted by the liver in a similar manner to bilirubin (Dragstedt and Mills 1936 Rosenthal and White 1925) the results of the bromsulphalein test also support this view The conclusion concerning the period of maximum liver damage, made on the basis of the results of the H A test, is consistent with the findings during the pre icteric stage it has already been shown that the discrepancy between the serum bilirubin curve and the improvement in H A synthesis is not so great if the rate of increase of the serum bilirubin concentration is taken as the measure of bilirubin excretory power instead of the actual serum level

It is much less easy to express an opinion on the progress of liver repair during convalescence Our own investigations, as far as they go, suggest a rapid recovery in most cases to full function But there is much evidence that liver damage can persist, at least in some cases, for many months, and the problem awaits further elucidation

A schematic summary of the biochemical course of the disease is given in Fig 16 It has been drawn from the study of different stages in different individuals and is only intended as a probable approximation In parti

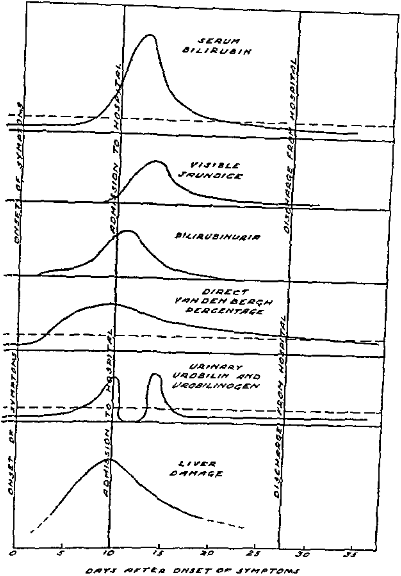


FIG 16 *Diagrammatic Summary of the Course of an Average Case of Infective Hepatitis a Composite Picture based on Results obtained from Different Tests at Various Stages of the Disease in nearly 300 Cases*

(Upper limits of normal, if not coincident with the base line, are roughly indicated by the broken horizontal lines)

the curve of "liver damage" must be regarded merelv as a reasonable guess based on rather limited evidence From a biochemical point of view the disease appears to be primarily an acute affection of the liver in which the liver becomes involved not long after the onset of symptoms Liver dysfunction rapidly increases until in most cases, the reserve for bilirubin excretion

rubin excretory reserve is never exhausted and no rise in serum bilirubin ever occurs The diagnosis is likely to be delayed in most cases until jaundice is apparent, i e until the liver is well on the way to spontaneous recovery, unless special efforts are made to detect the disease in the pre icteric stage Sub-icteric cases may be missed altogether Specific treatment can hardly be expected, therefore, to have any striking effect unless cases are diagnosed some time before the appearance of jaundice. The greatest practical contribution that biochemistry can make to the treatment of infective hepatitis therefore is to assist in early diagnosis

References

BARKER, H M, CAPPS R B and ALLEN, F W (1945a) *J Amer med Ass*, 128, 997

——— ——— ——— (1945b) *Ibid*, 129 653

402

, 34 467

I 127

IV. HAEMATOLOGICAL INVESTIGATIONS

by

J A R MILES M B

INVESTIGATIONS of the blood picture in infective hepatitis (usually under the name of catarrhal jaundice) have been reported from time to time since the beginning of this century but the figures given of the early investigations strongly suggest that cases of leptospiral jaundice were included The first paper giving the results of a considerable investigation of the blood picture after the differentiation of epidemic infective hepatitis by Cockayne (1912) is that of Jones and Minot (1923), who give a detailed account of changes in both white and red counts Thewlis and Middleton (1925) made a further investigation and since that time various other accounts have been published

In the present investigation the results of white cell counts on 30 cases from various sources seen before the onset of jaundice are reported and the results have been analysed with special reference to the value of the blood picture in early diagnosis A follow through of the white cell count in the first 80 adult male military cases seen before the fourteenth day of the disease and observations in the acute phase of others are described while various observations on the trend of the haemoglobin level and erythrocyte count ... erythrocyte

Control

Methods

Except in pre icteric or other cases in which the diagnosis was in doubt when blood was collected as soon as possible venous blood was taken from patients after breakfast between 9 30 and 11 00 a m the blood counting pipettes being immediately filled directly from the needle and films made on 3 × 1 in glass slides in such a way as to give free edges on either side The differential count was done on 300 cells except where it was not possible to find so many, by a 'battlement' count as advocated by Macgregor Richards and Loh (1940) Haemoglobin estimations were done by the usual Haldane technique using a N P L calibrated instrument The accuracy of haemo

of day and by the same technique on individuals who had take

exercise that day and who had in most cases been sitting down for at least 15 to 30 minutes before they were bled Further counts on 125 normals (mostly young men) were analysed to find out how often plasma cells and other abnormal types of cell appeared and to discover how many might be present in any one film These latter counts were taken at any time of day and not necessarily under conditions of rest, they include the 21 controls analysed more fully

Sternal punctures were made with a Salah needle 1 in below the junction of the manubrium with the body of the sternum approximately 0 2 ml of marrow was removed and films were made directly from this material differential counts were done on 500 cells The activity of the marrow was judged from the fattiness apparent cellularity and the proportions of various types of cell No total cell counts were made

Sedimentation rates were measured in Wintrobe tubes using the ammonium-potassium oxalate mixture advocated by Wintrobe and Landsberg (1935) and the correction charts for variation in the percentage volume of packed R B C made by Whitby and Hynes (1938) The volume of packed R B C. was obtained by spinning to constant volume at 2,000 r p m which in a group of 18 normals gave an average mean corpuscular volume of 87 c μ

Leucocytes

The description of the results of white cell counts has been separated into a description of the pre icteric and icteric phases, since the material investigated in these two phases is not comparable

PRE-ICTERIC PHASE

Infective hepatitis differs from the majority of infectious diseases in showing its most characteristic blood changes early in the disease and by beginning to return towards normal by the time icterus becomes apparent clinically

Eighteen of the cases considered here occurred in an outbreak in a mental institution, a further 8 came from various civilian outbreaks and only 4 were in Service personnel Sixteen cases were females and 10 were children between the ages of 7 and 15 The figures of the various cases did not show any differences between these small groups

Details of the individual counts on pre-icteric cases and sub-icteric cases [illegible] ...ilirubin are given in [illegible] ...es for the means and [illegible] Table 37, compared with the means of a group of 21 control counts done using the same technique

Total White Blood Cell Count

The total white cell count of 23 pre icteric and early sub-icteric cases when first seen varied between 8,400 and 2,700 per c mm with a mean of 4,790 as against 6,530 in the group of 21 normal controls These controls are not strictly comparable with the pre icteric cases, and statistical analysis is not justifiable No less than 17 of the 23 counts were under 5,000 Only 4 of the 23 cases had more than one total count during the pre-icteric phase and they do not show any clear-cut trend [illegible]

in the pre icteric phase had they been seen equally early

Polymorphonuclear Neutrophils

In the same series of total counts the mean total neutrophil count was 2 430 per c mm, with limits of 6 040 and 920 Eighteen of these counts were below 3,000 The mean figure for the controls was 4,120 per c. mm The mean percentage of neutrophils in 30 differential counts was 51 (as against 64 in controls) with extremes of 82 and 27 Twenty three of these showed less than 60 per cent. neutrophils and 17 less than 50 per cent

The total neutrophil counts of the 3 cases showing over 70 per cent neutrophils (Mi 3, C 3 and EC 4) were 6 040, 3,450 and 3,270 per c mm, respectively The first of these patients had Flexner dysentery as a complication and the second had an onset with sore throat.

TABLE 37

idıvidual Counts in Pre icteric Cases and Sub icteric Cases seen near the Onset of Symptoms

Total WBC (per c mm)	Neutrophils		Lymphocytes		Monocytes		Eosinophils		Basophils		Abnormal leucocytes	
	Number (per c mm)	Percentage of total WBC	Number (per c mm)	Percentage of total WBC	Number (per c mm)	Percentage of total WBC	Number (per c mm)	Percentage of total WBC	Number (per c mm)	Percentage of total WBC	Number (per c mm)	Percentage of total WBC
800	2 300	49	1 790	37	380	8	190	4	20	<1	60	1
300	3 270	76	640	15	290	7					120	3
600	2 300	50	1 490	32	580	13	?0	<1			230	5
		51		30		13		4				3
		44		38		12		1				5
6 600	2 800	42	2 860	43	750	11	130	2	?0	<1	110	2
		57		20		12		3		<1		9
3 850	1 560	41	1 500	38	480	13					3 0	10
		61		32		4		<1				3
[illegible]00	1 890	49	1 090	29	550	15	70	2			200	5
	2 200	46	1 840	38	620	13	80	2	20	<1	30	<1
	920	27	1 720	49	390	11	40	1			440	13
0	1 720	38	1 950	43	680	15	30	<1	10	<1	100	2
	2,540	65	990	25	260	7	30	<1	10	<1	80	2
4 000	1 560	39	1 760	44	520	13					160	4
7 400	3 000	40	3 100	42	840	11	70	1			420	6
		38		35		15		4		<1		7
6 400	2 930	46	2 810	44	380	6	50	<1			220	4

Mi 1	M 19	1	2,700	1 290	48	890	33	260	10	50	2	40	2	170	7
Mi 2	M.24	10	3 800	1 580	42	1 220	32	680	18	90	3	20	<1	210	6
		9	4 900	960	20	2 450	50	850	18	150	3	20	<1	470	10
		6	4 700	2 120	51	1 300	31	580	14	100	3			100	3
		3	4 000	1 540	39	1 540	39	740	19	40	1	40	1	100	3
Mi 3	M 30	8	7 700	6 040	79	1 000	13	500	7			40	<1	120	2
"		5	4,700	2,860	61	1 200	26	520	11	70	2			50	1
		3	7 600	4 790	63	1 790	24	680	9	150	2	80	1	110	2
		2	8 800	5 630	64	2,200	25	530	6	90	1	40	<1	310	4
C 3	F 17	4	4 200	3 450	82	570	14	80	2	20	<1			80	2
		2	3 100	1 430	46	1 250	41	280	9					120	4
C 4	F 18	6	3 400	1 970	58	1 170	35	240	7					20	<1
		4	2 250	1,220	54	690	31	260	12					90	4
EC 15	M 14	Sub-icteric			35		47		15		1		<1		1
EC 16	F.27				70		23		4		1				1
EC 17	M 13		4 700	2 980	61	1 420	30	280	6	10	<1	10	<1	90	2
EC.18	F 34		4 500	2,250	50	1 300	29	670	15	50	1	50	1	180	4
C 6	F 21		3 600	1 900	53	1 300	36	360	10					40	1
					53		36		7				1		3
C. 7	F 7		8 400	3,280	39	3 780	45	840	10	250	3			250	3
C 8	M 10		4 800	1 950	41	1 390	29	1 100	23	170	4	50	1	140	3

EC — Institutional outbreak C = Other civilian cases Mi = Military cases

Percentages are given to the nearest whole number

In addition to the neutropenia there was a transient shift to the left in the neutrophils with an increase in unsegmented cells and with the occasional appearance of myelocytes in the peripheral circulation The following count taken on the 8th day before the onset of jaundice, the day after the onset of symptoms, is typical total W B C 4,600 per c mm , neutrophils 50 per cent (2,300) divided into unsegmented cells 19 per cent and segmented 31 per cent In the film showing the greatest shift to the left, unsegmented neutrophils made up 38 per cent of all white cells while segmented forms were only 15 per cent This increase in unsegmented neutrophils had usually disappeared within one or two days after the onset of jaundice

Lymphocytes

The percentage of lymphocytes in the majority of pre icteric cases was above the commonly accepted normal limits, although the mean total figure of 1,630 per c mm. was below that of 1,930 in the controls The limits were 3,780 to 570 per c mm , 2 cases showing counts above 3 000 and 4 below 1,000

There was, however, a very definite shift towards a predominance of young lymphocytes, with an absolute increase in large lymphocytes and an absolute decrease in small lymphocytes In this series of cases the ratio small large lymphocytes was 2 75 1 (limits 4 3 to 1 3) as against a normal of 3 82 1 (limits 6 5 to 3) found by the same observer More than usual of the large lymphocytes showed remnants of nucleoli and sometimes typical lymphoblasts were seen, although cells were not diagnosed as such so often as by Thewlis and Middleton (1925) The large lymphocytes in most cases contained numerous azurophil granules and in a few these were very large (up to 2 μ in diameter) The increase in young lymphocytes was less transient than that in neutrophils, and its course is described further on p 95 The mean lymphocyte percentage was 33 (controls 29) with limits of 49 to 13 In 21 of these 30 pre icteric and sub icteric cases the percentage was over 30, while in 3 only was it below 20

Monocytes

The monocytes like the lymphocytes were relatively more numerous in the pre icteric cases than in the controls and the mean figure of 11 per cent (controls 6 per cent) was the highest found at any stage of the disease but the mean total count of 430 per c mm was hardly different from that of the controls (400 per c mm) and much ' than the figure of 580 per c mm in the earl

The total figures varied ⁰⁰ and 80 while the percentages extended from 10 per ytes were present in 19 of the 30 normal as seen in Leishman and G is but a ss cyto plasmic vacuolation. This most in there marked rise in

E ' *Basophils*

between

cou between 2

a hepatitis

TABLE 38

Pre icteric and Early Sub icteric Cases Means and Extremes of 23 Total W B C Counts compared with 21 Control Total W B C Counts*

Group	Count	Total W B C (per c mm)	Neutrophils (per c mm)	Lymphocytes (per c mm)	Monocytes (per c mm)	Abnormal leucocytes (per c mm)
23 pre-icteric and sub-icteric cases	Mean	4 790	2,430	1 630	430	170
	Maximum	8 400	6 040	3 780	1 100	440
	Minimum	2 700	920	570	80	20
21 normal controls	Mean	6 530	4 120	1 930	400	occasional
	Maximum	11 500	6 550	4 330	880	30
	Minimum	4 500	2 760	1 120	170	0

* Total W B C counts were only available for 23 of the cases shown in Table 37

TABLE 39

Pre-icteric and Early Sub icteric Cases Means and Extremes of 30 Differential W B C Counts compared with 21 Control Differential W B C Counts

Group	Count	Percentage of			
		Neutrophils	Lymphocytes	Monocytes	Abnormal leucocytes
30 pre-icteric and sub-icteric cases	Mean	51	33	11	4
	Maximum*	82	49	23	13
	Minimum*	27	13	2	<1
21 normal controls	Mean	64	29	6	<1
	Maximum*	70	38	10	<1
	Minimum*	55	19	3	0

* Maximum and minimum percentages were observed figures for each type of cell
Percentages are given to the nearest whole number

Abnormal Cells

The abnormal white cells seen in infective hepatitis unlike those in infectious mononucleosis are mainly of one type This cell is a large plasma cell with a deeply basophilic, slightly granular and vacuolated cytoplasm and usually with a typical eccentric cartwheel nucleus As is usual in plasma cells in the peripheral blood there is somewhat less cytoplasm than in similar cells found in the tissues or the bone marrow Following the view of Moeschlin (1940) that plasma cells develop directly from plasmoblasts and that the cells commonly called Turck cells are young plasma cells, cells with a similar cytoplasm but a nucleus showing remnants of nucleoli and not showing the typical cartwheel arrangement of chromatin have been included as plasma cells In some cases there are a few Downey type I

cells, the most common type in infectious mononucleosis, but in none has there been any difficulty in differentiating the blood picture The average number of abnormal cells was 170 per c mm with limits of 440 to 20 while the mean percentage was 4 (limits 13 to <1) In a series of 125 differential counts on normal individuals abnormal cells forming as much as 1 5 per cent of the total white cell count were seen once lower percentages in 20 counts and none in the remainder Two per cent. of the total white cell count has, therefore, been taken as the upper limit for abnormal white cells in normal individuals Eighteen of the 30 pre icteric and sub icteric cases showed a higher percentage than this in their first count Of the 12 cases which showed 2 per cent or less in their first count, 4 had more than one count in the pre icteric phase, all these showed more than 2 per cent in a later count.

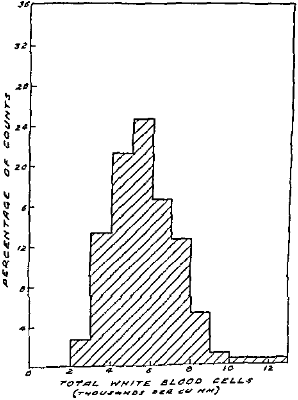


FIG 17 *Distribution of Counts in First Counts of Total W.B C (150 cases)*

ICTERIC PHASE

The first counts on Service cases seen after jaundice had developed, but less than 14 days from the first symptom, have been analysed in two ways Firstly distribution curves of the total white cell count, and of the total counts of the main types of cell, have been plotted in an attempt to demonstrate any subsidiary peaks suggestive of separate groupings with either a higher or a lower count than that of the main body Secondly cases in which the disease was acquired in the Middle East have been compared with cases among troops from the Home Forces

Distribution of White Cell Counts in Early Cases

In this analysis the first counts on 150 cases seen less than 14 days after the onset of symptoms have been treated as one group and plotted as frequency distribution diagrams in Figs 17 to 20

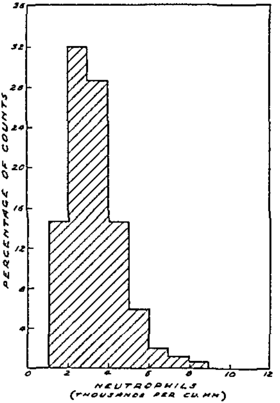


FIG 18 *Distribution of Counts in First Counts of Total Neutrophils* (150 cases)

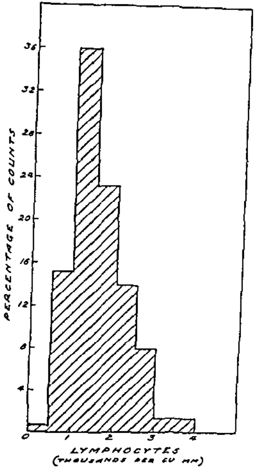


FIG 19 Distribution of Counts in First Counts of Total Lymphocytes (150 cases)

TABLE 40

First W B C Counts of 150 Service Cases Seen After the Onset of Jaundice Compared with Controls

Group	*Count*	*Total W B C (per c mm)*	*Neutrophils (per c mm)*	*Lymphocytes (per c mm)*	*Monocytes (per c mm)*
Service cases with jaundice	Mean	5 690	3 300	1 610	570
	Maximum	12 100	8 470	3 720	1 200
	Minimum	2 400	780	460	200
Controls	Mean	6 530	4 120	1 930	400
Standard error of difference		398 5	304 5	166	22 1
D/S E		2 1	2 7	1 96	7 86

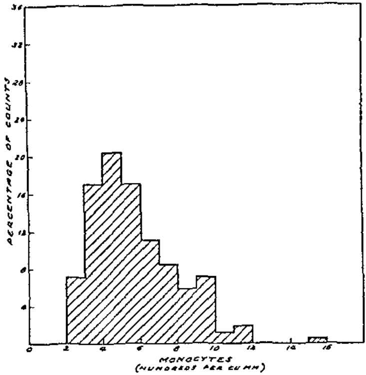


FIG 20 *Distribution of Counts in First Counts of Total Monocytes* (150 cases)

In the total white cell count and in those of all the main types of cell there is a slightly skew curve with a tail of high counts The range of the count is slightly wider than that of the control group in the total and neutrophil counts, but the range of the lymphocyte count differs little from that of the control group (Table 38, p 89) The monocyte count shows a much wider spread than in the controls The curves fail to show the secondary peaks which might be expected if the cases had not belonged to a homogeneous group

The means and extremes of the counts are tabulated in Table 40 and compared with the controls The general leucopenia and neutropenia may be regarded as significant (D/S E 2 1 and 2 7 respectively), while the lymphopenia (D/S E 1 96) is below the usually accepted level of significance The monocytosis (D/S E 7 86) is highly significant

The mean total of 10 first counts on adult women with infective hepatitis was 4,700 per c mm., as against 5,690 for the men, with a very similar differential count to that of the men No conclusions, however, can be drawn from so small a group

Comparison of White Cell Counts in Middle East and Home Force Cases

In Tables 41 and 42 the results of total white cell and differential white cell counts on cases of infective hepatitis among men recently returned from the Middle East are compared with those in Home Force troops The cases in troops returned from the Middle East are divided into cases developing less than one month and more than one month after landing in the United Kingdom All these counts were done on the morning following admission to hospital and only counts done less than 14 days after the onset of symptoms are included The Tables show a very close agreement between the three groups of patients Table 41 shows the total counts and Table 42 the percentage counts

In 93 of the 114 counts considered here plasma cells were present in numbers varying from 1 seen in a count of 300 cells to 9 3 per cent In 19 of the counts more than 2 per cent of plasma cells were present There was no gross difference in the numbers of these cells between the various sub groups

TABLE 41

Comparison of First W B C Counts (Totals) of Cases of Infective Hepatitis from Middle East Forces with Those from Home Forces

Group		*M E F (A)*	*M E F (B)*	*H F*
Number of cases		27	30	57
Total W.B C (per c mm)	Mean	5 720	5 760	5 670
	Maximum	11 100	8 700	9 800
	Minimum	2 600	3 100	2 700
Total neutroph ls (per c mm)	Mean	3 370	3 480	3 300
	Maximum	7 700	5 600	6 660
	Minimum	1 540	1 430	1 270
Total lymphocytes (per c mm)	Mean	1 660	1 480	1 710
	Maximum	3 720	3 130	3 380
	Minimum	620	460	710
Total monocytes (per c mm)	Mean	550	610	580
	Maximum	960	1 200	1 100
	Minimum	270	200	260

M E F (A) Middle East Forces onset of infective hepatitis w thin 1 month of arrival in England
M E F (B) Middle East Forces onset later than 1 month after arrival
H F Home Forces

The Course of the White Cell Counts

The total and differential white cell counts have been followed throughout the course of the disease in 80 Service cases and 40 of these had a further blood count at a follow up approximately one month after their discharge from hospital

The counts of all the main types of cell showed definite trends during the course of the disease these are shown in Table 43 In the first 18 days after the onset the mean total of all W B C and of neutrophils varied little from that shown for the first counts on 150 cases The plasma cells fewer in the earliest icteric cases than in the pre icteric fell rapidly

TABLE 42

Comparison of First W B C Counts (Percentages) of Cases of Infective Hepatitis from Middle East Troops with Those from Home Forces

Group		M E F (A)	M E F (B)	H F
Number of cases		27	30	57
Neutrophils (per cent of total W B C.)	Mean	56	60	56
	Maximum*	79	80	76
	Minimum*	30	39	33
Lymphocytes (per cent of total W B C.)	Mean	30	26	30
	Maximum*	58	45	57
	Minimum*	13	9	13
Monocytes (per cent of total W.B C)	Mean	10	11	10
	Maximum*	16	19	23
	Minimum*	6	5	4

M E F (A) Middle East Forces onset of infective hepatitis within 1 month of arrival in England
M E F (B) Middle East Forces onset later than 1 month after arrival
H F Home Forces

* Maximum and minimum percentages were observed figures for each type of cell
Percentages are given to the nearest whole number

TABLE 43

Means of W B C Counts at Different Stages in the Disease in 80 Service Cases followed throughout their Course

Days from onset of symptoms	Number of readings	Total W B C (per c mm)	Neutrophils (per c mm)	Lymphocytes (per c mm)	Monocytes (per c mm)	Abnormal leucocytes (per c mm)
<7	40	5 850	3 330	1 730	580	108
8—10	48	5 610	3 160	1 610	510	65
11—14	72	6 030	3 330	1 840	550	65
15—18	57	5 690	3 160	2,000	460	38
19—21	35	6,100	3 480	1 950	510	23
22—28	62	6 300	3 590	2 040	490	not calculated
29—35	50	6 580	3 690	2 100	460	
36—49	35	6 590	3 850	2 130	430	
50+	40	7 460	4 690	2 150	480	

from an average of 108 per c mm in cases seen before the 7th day after the onset of symptoms to 65 per c mm between the 8th and 10th days and numbers greater than those which may be found in normal individuals were not seen after the 18th day Over the same period the number of lymphocytes rose in such a way as to cancel the fall in monocytes and plasma cells

From the 18th day onwards the numbers of neutrophils and the total count began to rise By the 5th week from the onset the total count had reached the level of the controls but the number and percentage of neutrophils was lower than normal The lymphocytes at this stage were raised above normal both in percentage and absolute numbers The ratio of small to large lymphocytes in the first 10 days of the disease was the same in the icteric as in the pre icteric cases After this it slowly returned to normal the

Erythrocytes

Erythrocyte counts and haemoglobin and haematocrit estimations were made in all cases, including chronic and relapsed cases, on admission, and the two latter estimations were repeated at intervals throughout the course of the disease On the rare occasions when a haematocrit reading below 40 per cent was obtained another complete blood count was performed The erythrocyte count and haemoglobin level were followed in 14 unselected cases as well as in 2 cases which showed anaemia In the unselected cases no development of anaemia was found and the mean results of the counts on admission and during recovery are shown in Table 44 The haemoglobin level and the erythrocyte count were unchanged, while the haematocrit figure and mean corpuscular volume were slightly lower in convalescence. The M C V was in both slightly above that in the controls (87 c μ) done by the same technique with the same apparatus

None of the cases which were admitted late in the disease, except for Bdr M, discussed below, showed any anaemia The only other case to show definite anaemia developed it during his stay in hospital

TABLE 44

Mean Results of Erythrocyte Counts and Haemoglobin and Haematocrit Estimations in 14 Unselected Cases of Infective Hepatitis, on Admission and During Recovery

Time	R B C count (millions per c mm)	Haemoglobin (Per cent Haldane)	Haemoglobin (Grammes per cent)	Haematocrit (per cent)	Mean corpuscular volume (c μ)
On admission	5 28	104	14 4	49 4	93 6
During recovery	5 17	104	14 4	46 4	89 7

Bdr M was admitted about 3 weeks after the onset of symptoms and despite moderate jaundice his conjunctivae appeared abnormally pale His jaundice was at that time beginning to fade His R B C numbered 3 47 millions per c mm and his haemoglobin was 72 per cent (9 9 g Hb per cent) giving a colour index of 1 04 while a haematocrit reading of 33 per cent gave a mean corpuscular volume of 95 c μ Two days after his admission he received an injection of 3 ml of "Hepatex"

The type of anaemia with a slightly raised M C V and colour index, and the recovery without therapy rapidly following on the disappearance of jaundice suggest that this anaemia was consequent upon the hepatitis

The second case, Cpl S developed a slight degree of anaemia during his stay in hospital his haemoglobin falling from 94 to 80 per cent (13 0 to 11 0 g Hb per cent.) and his R B C. count from 4 76 to 3 6 millions per c mm His jaundice was mild and at this stage of his disease he was discharged unfortunately it was not possible to see him again

absolute number of small lymphocytes reaching normal in the 3rd week while the ratio of small to large lymphocytes had usually returned to normal by the 6th or 7th week The progress of this ratio in the first 50 cases in this series is shown in Fig 21 The monocytes continued to fall until the lowest recorded mean of 430 per c mm, was found in the 6th and 7th weeks

All figures in the follow-up were high, possibly because nearly all the patients had travelled considerable distances before they were seen but this was the only group in which the percentages in the differential count approached normal (neutrophils 62 per cent, lymphocytes 29 per cent., monocytes 7 per cent)

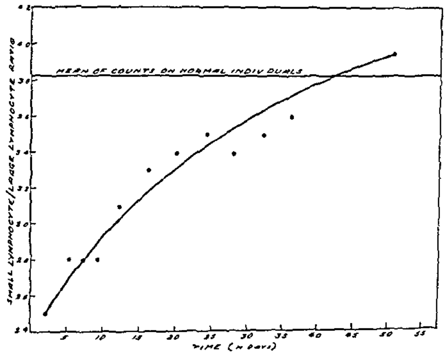


FIG 21 *Variation of the Ratio of Small to Large Lymphocytes with Time and a Curve of the Trend*

The White Cell Count during Relapse

When a relapse of the clinical and biochemical condition occurred there was also a return of leucopenia, with a fall in the total neutrophil count and usually an increase in the relative neutropenia with much smaller and less regular variations in the total lymphocyte count There was little change in the type of cell during the relapse and a rise in plasma cells to more than 2 per cent was most unusual

Similar haematological changes occasionally occurred without any signs of clinical or biochemical relapse or the blood picture became steadily more abnormal for some time despite rapid clinical recovery

Erythrocytes

Erythrocyte counts and haemoglobin and haematocrit estimations were made in all cases, including chronic and relapsed cases, on admission, and the two latter estimations were repeated at intervals throughout the course of the disease On the rare occasions when a haematocrit reading below 40 per cent was obtained another complete blood count was performed The erythrocyte count and haemoglobin level were followed in 14 unselected cases as well as in 2 cases which showed anaemia In the unselected cases no development of anaemia was found and the mean results of the counts on admission and during recovery are shown in Table 44 The haemoglobin level and the erythrocyte count were unchanged, while the haematocrit figure and mean corpuscular volume were slightly lower in convalescence The M C V was in both slightly above that in the controls (87 c μ) done by the same technique with the same apparatus

None of the cases which were admitted late in the disease, except for Bdr M, discussed below showed any anaemia The only other case to show definite anaemia developed it during his stay in hospital

TABLE 44

Mean Results of Erythrocyte Counts and Haemoglobin and Haematocrit Estimations in 14 Unselected Cases of Infective Hepatitis, on Admission and During Recovery

Time	*R B C coun. (millions per c mm)*	*Haemoglobin* (Per cent Haldane)	*Haemoglobin* (Grammes per cent)	*Haematocrit (per cent)*	*Mean corpuscular volume (c μ)*
On admission	5 28	104	14 4	49 4	93 6
During recovery	5 17	104	14 4	46 4	89 7

Bdr M was admitted about 3 weeks after the onset of symptoms and despite moderate jaundice his conjunctivae appeared abnormally pale His jaundice was at that time beginning to fade His R B C numbered 3 47 millions per c mm and his haemoglobin was 72 per cent (9 9 g Hb per cent.) giving a colour index of 1 04 while a haematocrit reading of 33 per cent gave a mean corpuscular volume of 95 c μ Two days after his admission he received an injection of 3 ml of "Hepatex"

The type of anaemia with a slightly raised M C V and colour index, and the recovery without therapy rapidly following on the disappearance of jaundice suggest that this anaemia was consequent upon the hepatitis

The second case, Cpl S., developed a slight degree of anaemia during his stay in hospital, his haemoglobin falling from 94 to 80 per cent. (13 0 to 11.0 g Hb per cent) and his R B C. count from 4 76 to 3 6 millions per c mm His jaundice was mild and at this stage of his disease he was discharged unfortunately it was not possible to see him again

In the ordinary mild or moderate case of infective hepatitis target cells were seen only rarely and in 10 unselected cases at the height of icterus or early in recovery the erythrocyte fragility was within normal limits A few severe or chronic cases showed increased numbers of target cells and the case with the largest number of these cells (7 per cent) showed only 95 per cent haemolysis in 0 24 per cent saline and complete haemolysis in 0 20 per cent saline Numbers of target cells as high as those reported by Greenblatt and Kaplan (1943) in post vaccinal hepatitis were not seen and as would be expected increased resistance to lysis by hypotonic saline was correspondingly rare

Sternal Marrow

Sternal punctures were done on 11 cases of infective hepatitis on one case of acute necrosis of the liver of unknown origin 4 hours before death and on one case of acute necrosis of the liver following arsenotherapy 24 hours before death

In the non fatal cases except for Bdr M discussed above who showed hyperactivity there was no abnormality in the cellularity of the marrow nor was there any abnormality in the proportion of the different types of cell seen except for the presence of more plasma cells than usual in nearly all cases The average number of plasma cells was 1 97 per cent. of all cells different counts varying from 4 2 to 0 8 per cent Osgood and Seaman (1944) analysing 473 counts on normals by various authors give a mean normal of 0 51 per cent plasma cells In most cases at the time of sternal puncture the number of plasma cells found in the sternal marrow was greater than that in the peripheral blood Generally the plasma cells in the marrow had very large amounts of cytoplasm more than in the majority of those in the peripheral blood

In the marrow films from the 2 fatal cases of acute necrosis there were signs of hyperactivity in the marrow and many of the myelocytes showed marked cytoplasmic vacuolation Hyperactivity of the marrow is usual in fatal cases and Lucké (1944) has suggested that it is related to the haemorrhagic phenomena which are a very constant finding at post mortem

TABLE 45

Mean Erythrocyte Sedimentation Rates at Different Levels of Serum Bilirubin

Serum bilirubin (mg per 100 ml)	*Number of readings*	*Mean E S R (mm in first hour)*
>10	20	5 30
8 1—10	18	8 39
6 1—8	12	8 0
4 1—6	18	10 56
2 1—4	29	14 31
<2	34	18 94

Erythrocyte Sedimentation Rate

The E S R was raised at some stage of the disease in one half of the 80 cases studied The rise in the E.S R nearly always occurred when the serum bilirubin was either falling or approaching normal but in 6 out of 8 pre icteric cases it was raised and in 2 of these out of 3 on which later

estimations were made, the E S R fell when icterus developed When the mean figures for the E S R at various bilirubin levels were tabulated it was found that the mean figures rose fairly steadily as the bilirubin fell (Table 45) There was a highly significant negative correlation between the level of the serum bilirubin and the E.S R (131 readings coefficient of correlation − 0 57)

Experiments with blood from 8 cases of various diseases with no obvious liver damage but with a raised E S R, showed that in each case the E S R could be brought back towards normal if a solution of crude bile salts was added to the blood and the mixture stored for 1 to 4 hours before the estimation was made (Table 46) The addition of bilirubin in amounts up to 10 mg per cent had no effect Similarly Bendien Neuberg and Snapper (1932) have reported slowing of the E S R following the addition of cholic acid

These findings are reported in detail and more fully discussed elsewhere (Miles 1945)

TABLE 46

Effect of the Addition of Bile Salts and Storage on the E S R

Amount of bile salt added to plasma (mg per 100 ml)	*Corrected E.S R (mm in first hour)*	
	After storage for 1 hour	*After storage for 4 hours*
0	30	20
12 5	29 5	10
25	20	8
50	1	1

Discussion

PRE ICTERIC STAGE

Certain general features in the white cell count in fully developed infective hepatitis seem to be generally agreed, but in the pre icteric phase where each observer has seen fewer cases than in the icteric there is less agreement Jones and Minot (1923) held that in the pre icteric stage there was a preliminary leucocytosis lasting about 3 days followed by a leucopenia Thewlis and Middleton (1925) who disagreed with this finding pointed out that their later figures were lower than those in the pre icteric state of the disease, but that the total counts even then were not higher than normal Ziegler (1941) found a lowered or low normal total leucocyte count and a reduced total polymorph count with a large percentage increase in "stab" cells More than half our own material on the pre icteric phase was from a single outbreak in a mental institution the remaining 12 cases represent at least seven different outbreaks This heterogeneous collection of material shows a total white cell count of under 5 000 per c. mm in 17 out of 23 counts and a total neutrophil count below 3 000 per c mm in 18 out of 23 counts, i.e. a definite leucopenia and neutropenia in the great majority of cases No clear-cut trend in the counts during the pre icteric phase can be seen although examination of the figures in Table 37 (p 86) [illegible]

Most observers are agreed that although the lymphocyte percentage may be above normal in the pre icteric stage the total count is usually below normal and the present findings agree with this view On the other hand, in these cases, although the percentage of monocytes was very high, the total monocyte count was but little above normal

Most of those who have discussed the blood picture in infective hepatitis have mentioned the appearance of abnormal cells Jones and Minot stated that the presence of immature and abnormal white cells was a feature of the condition and stated that definite plasma cells were also present They observed vacuolation of the leucocytes, especially of the monocytes Thewlis and Middleton noted an increase in the number of young types of lymphocyte and the presence of some lymphoblasts Dunlop (1932) and Findlay, Dunlop and Brown (1931) refer to an increase in large lymphocytes and the presence of "glandular fever cells" Ziegler (1941) records the presence of up to 10 per cent of plasma cells in the early stages of the disease and Buerger (1943) reports an incidence of 2 to 5 per cent of plasma cells Barker *et al* (1945) describe the abnormal cells found in cases in the Mediterranean area as atypical lymphocytes similar to those of glandular fever In our cases there was an increase in the number of large lymphocytes present (with lymphoblasts occasionally) and a decrease in the number of small lymphocytes while the proportion of plasma cells varied from <1 to 13 per cent Out of 30 first counts 18 showed over 2 per cent of plasma cells The typical abnormal cells of glandular fever were only occasionally seen and then in very small numbers

Usually infective hepatitis can readily be differentiated from infectious mononucleosis with biluria by the lack of the typical glandular fever cells and the presence of leucopenia During this investigation several cases were seen in which blood films suggested infectious mononucleosis, and in all of them the clinical picture suggested the same diagnosis and the Paul Bunnell reaction was positive

Certain other conditions which are liable to become confused with the infective hepatitis prodrome are not so easily distinguished haematologically Rubella often shows a very similar picture (Hickling, 1925, Hynes, 1940, Moeschlin, 1940) although neutropenia is not so common and the number of plasma cells is usually greater Hynes, who defined plasma cells very strictly as the typical adult type with eccentric, cartwheel nucleus, and called all other similar cells Turck cells, found only a small percentage of typical plasma cells and much larger numbers of Türck cells in rubella Had the same differentiation been used in this series the relative proportion of Turck [illegible]

hepatitis

Primary attacks of malaria are often very difficult to differentiate clinically, especially as the parasites may be extremely scanty The white cell picture at least as seen in England, is sometimes exactly the same as that in infective hepatitis, although the percentage of neutrophils is usually higher Further, the statement commonly made that a monocyte count of over 15 per cent

Where the alternative diagnosis is a condition in which an absolute neutrophil leucocytosis is to be expected, the white cell count can be of great value The absence of leucocytosis in a patient whose disease begins with acute abdominal pain may save him from a laparotomy, and the presence of leucocytosis in a case suspected of Weil's disease will go far to establish the diagnosis

ICTERIC STAGE

After the appearance of jaundice the white cell count usually showed a gradual return towards normal in the Service cases which were followed, in most cases when first seen and had always returned to normal within the total and neutrophil counts beginning to rise on the average about the 18th day and being back to normal 4 to 5 weeks after the onset of symptoms The proportion of mature to immature neutrophils was normal 14 days of the onset of symptoms

The total number of lymphocytes began to increase after about the 10th day and the increase was due to an increase in the numbers of small lymphocytes By the 15th to 18th day the number of small lymphocytes had returned to normal while the number of large lymphocytes remained increased, both the relative and total lymphocyte counts were above the average of controls throughout convalescence In measles Naegeli (1931) describes similar changes in the lymphocyte count, namely lymphopenia in the acute stage followed by a lymphocytosis in convalescence In infective hepatitis the ratio of small to large lymphocytes had usually returned to normal by the 6th week from the onset In the military cases there was both a relative and absolute monocytosis, greatest in the earliest cases and steadily returning towards normal, but neither the mean percentage nor the mean total of monocytes had fallen to the level of the controls even 7 weeks after the onset In many of the early cases vacuolation of the monocytes as described by Jones and Minot (1923), was observed Tompkins and Cunningham (1938) found both monocytosis and abnormally large neutral red vacuoles in the monocytes in supravitally stained preparations, similar to those seen in other acute infections

The presence of an abnormal percentage of plasma cells was very transient and over 2 per cent was not found after the 18th day Ziegler (1941) found that these cells normally disappeared from the 7th to 14th day after the onset of icterus

The present series seems to be unusual in the lack of change in the haemoglobin level and the erythrocyte count. Jones and Minot (1923) reported a marked fall in erythrocyte count at the height of the disease, and Hallgren (1942) found a mean fall of 10 per cent. in the haemoglobin level during the course of the disease in an outbreak in a sanatorium in Sweden The fact that significant anaemia occurred in only 2 out of more than 80 cases in this series and was not present in relapsed and subacute cases admitted late in the disease suggests that previously healthy, well fed, young adult males do not respond in the same way as the populations seen by previous authors The more severe of the 2 cases which did become anaemic showed some macrocytosis but the sternal marrow showed normal maturation and there was no reticulocyte response to liver extract, his blood picture returned to normal without any treatment during convalescence

The mean corpuscular volume was usually above that of the normal con-

trols and is in line with the findings of Schalm (1937) and those of Ziegler (1941) who reported an increased R B C diameter without deformation of the corpuscle

Greenblatt and Kaplan (1943) reported the presence of up to 40 per cent of target cells in 22 cases of post vaccinal hepatitis with definitely increased resistance of the erythrocytes to hypotonic saline in 73 per cent of their cases In our series only occasionally in the most severe and chronic cases were significant numbers of target cells present and the erythrocytes of the usual mild or moderate cases did not show any increase in resistance to lysis by hypotonic saline

Buerger (1943) states that the bone marrow in his cases of infective hepatitis showed hyperactivity with an increase in the number of myelocytes plasma cells and normoblasts but he does not give any figures In our series no evidence was found of marrow abnormality in unselected cases except an increase in plasma cells In 1 anaemic case and 2 fatal cases there was evidence of hyperactivity

References

[illegible] *J Amer med Ass* **128** 997
eesk Bl **30** 1
[illegible] *Trans R Soc trop Med Hyg*
GREENBLATT I J and KAPLAN C (1943) *J Amer med Ass* **122** 806
HALLGREN R (1942) *Acta med scand* Suppl **140**
HICKLING R A (1925) *J Hyg Camb* **24** 120
[illegible] 9 531
[illegible] *J Path Bact* **51** 337
[illegible] 46
[illegible] **169** 159
[illegible] *Handbook of Haematology*
London
WHITBY L. E H and HYNES M (1938) *Lancet* ii 249
WINTROBE M M and LANDSBERG J W (1935) *Amer J med Sci* **189** 102
ZIEGLER N (1941) *Ann Paed* **157** 129

V. SEROLOGICAL INVESTIGATIONS

by

J A R MILES M B

THE possibility of finding a serological reaction for distinguishing different types of hepatitis has received some attention recently (Eaton, Murphy and Hanford, 1944, Findlay, Martin and Mitchell, 1944, Olitski and Bernkopf, 1945, Miles, 1946) The problems involved in differentiating between [illegible]

complement-fixation test which have been published elsewhere and an account of an investigation for the presence of heterologous agglutinins

Complement-Fixation Test

The techniques used in the preparation of antigens and in the execution of the complement-fixation tests are given in detail elsewhere (Miles, 1946) Livers were obtained from fatal cases of homologous serum hepatitis, infective hepatitis and arsenotherapy hepatitis Saline extracts of these, and of normal livers as controls, were used as antigens in complement-fixation tests against sera from both acute and convalescent cases of all these three conditions as well as against a variety of control sera

RESULTS

Acute Necrosis of Liver of Uncertain Aetiology

These livers all showed a histological picture similar to that of cases known to have been in contact with infective hepatitis Some of them gave extracts which were too anti-complementary for use Seventeen were usable, but none of them gave a greater degree of fixation with any sera than that obtained with control antigens made from normal livers

Fatal Cases of Infective Hepatitis Occurring During Epidemics

Antigens from 5 such livers were prepared Three were tested both against sera from cases occurring in the same outbreak (mainly relatives of the fatal case), and also against sera from other outbreaks of infective hepatitis and from cases of homologous serum hepatitis and arsenotherapy hepatitis The other 2 were tested against sera from all 3 types of hepatitis but not against any from cases of infective hepatitis from the same outbreak One of these latter antigens showed fixation with 5 out of 9 sera from convalescent cases of infective hepatitis these 5 sera gave no fixation with control antigens Positive fixation was not given with sera from cases of arsenotherapy hepatitis, which gave specific positive fixation with antigens prepared from livers of cases of the same condition Unfortunately only a very small quantity of this liver was available so that no more extensive investigations could be made The other liver antigens gave no fixation different from that obtained with antigens made from normal livers

Liver from Cases of Reputed Homologous Serum Hepatitis

Extracts from 9 such livers were tested against sera from known and reputed cases of homologous serum hepatitis and cases of infective hepatitis

and arsenotherapy hepatitis No complement fixation differing from that with control antigens was observed

Known Icterogenic Sera

These were highly anti complementary unheated and had to be heated to 56°C for 30 minutes before use Two batches of icterogenic serum were tested against sera from early and convalescent cases of homologous serum hepatitis resulting from their injection No complement fixation was found either with the untreated serum or with the supernatant or resuspended deposit of one specimen after centrifugation at 30 000 r p m for 30 minutes

Arsenotherapy Hepatitis

The results with liver antigens and sera from cases of arsenotherapy hepatitis are in marked contrast with the negative results obtained in infective hepatitis and homologous serum hepatitis

During the period in which the disease was epidemic in military clinics the serum in a high percentage of cases of arsenotherapy hepatitis contained a complement fixing antibody which reacted with liver extracts from cases dying of arsenotherapy hepatitis but not with any other liver extracts Sera collected earlier in the disease showed far fewer positives than those collected more than eight weeks after the onset of symptoms During the epidemic period 28 4 per cent of 67 sera collected before the eighth week gave fixation while 67 per cent of 55 sera collected after that time were positive A few sera gave fixation more than six months after the onset of the disease and 2 of 4 tested over a year after the onset gave fixation

The results of complement fixation tests made with 34 pairs of early and late sera from the same patients (before and after the eighth week) are shown in Table 47 Seven of these pairs were from cases which occurred after the epidemic incidence of arsenotherapy hepatitis had ceased and these together with 3 others tested only before the eighth week and not included in the Table all gave negative results The results with all the sera tested (including those recorded in Table 47) are shown in Table 48 In both Tables the sera are divided into those taken before and after eight weeks from the onset of the disease Where more than two samples of sera from any case had been tested only the results of the first and last tests have been used in compiling the Tables

TABLE 47

Results of Complement Fixation Tests in Arsenotherapy Hepatitis (34 pairs of early and late sera against liver from cases of arsenotherapy hepatitis)

Serum	Number negative	Number doubtful	Number positive	Total	Percentage positive
Early	28	0	6	34	17 6
Late	12	3	19	34	56

[illegible] hepatitis when complement [illegible] the second week In nearly [illegible] developed they were present by the eighth week but in one case none were found at that time but a titre of 1 40 had developed by the eleventh week

TABLE 48

Results of Complement-Fixation Tests in Arsenotherapy Hepatitis (all sera tested, including those in Table 47, antigen was liver from cases of arsenotherapy hepatitis)

Serum	Number negative	Number doubtful	Number positive	Total	Percentage positive
Early	54	4	19	77	24 7
Late	21	4	37	62	59 5

Seventy sera from 52 cases of infective hepatitis were also tested against liver extracts from cases of arsenotherapy hepatitis, 18 of these cases of infective hepatitis provided paired early and late samples of serum, and 16 of the samples of serum from the other 34 cases were from convalescent cases and 18 from acute cases None of these sera gave a positive complement fixation test.

Negative results were also obtained from 6 undoubted cases of homologous serum hepatitis and from a number of reputed cases of this condition Various types of control sera from normal subjects syphilitics (without jaundice) during their third course of arsenotherapy, syphilitics with a positive W R, cases of obstructive jaundice, and from patients with a variety of conditions showing liver damage, were also tested without finding any which gave similar results to those found with the sera of cases of arsenotherapy hepatitis (Table 49)

TABLE 49

Results of Complement Fixation Tests Using Control Sera against Antigens Prepared from Normal Liver and Liver from Cases of Arsenotherapy Hepatitis

Type of case	Number of sera showing no fixation	Number of sera positive with arsenotherapy hepatitis antigens only	Number of sera positive with all liver antigens	Total	Percentage of sera positive with all liver antigens
Normals	76	0	1	77	1 3
3rd course of arsenotherapy (without jaundice)	20	0	1	21	5
W R positive	12	0	1 (doubtful)	13	7 7
Obstructive jaundice	4	0	1	5	20
Miscellaneous conditions showing liver damage	17	0	3*	20	15

* Two of these sera were from cases of malaria (5 samples of serum from cases of malaria were tested in all)

It has been noted above that the sera from cases of arsenotherapy hepatitis did not always contain complement fixing antibodies Similarly the livers of cases dying of acute necrosis of the liver during arsenotherapy did not always yield the specific antigen of 16 such livers which were tested 10 showed antigenic activity differing from that of control livers

Nature of the reaction Attempts were made to elucidate the mechanism of the specific reaction between the livers of cases which had died of arsenotherapy hepatitis and the sera of acute and convalescent cases of the same disease Most of these attempts were based on the theory that the reaction was due to a complex formed of protein from damaged liver and arsenic compounds acting as an antigen However no experimental evidence was obtained in support of this theory

In an experiment of a different type a high titre antigen was spun in a Sharples super centrifuge at a speed and for a time sufficient to cause the deposition of a large part of any known virus After this treatment neither the supernatant nor the deposit showed any antigenic activity Dr R Markham who carried out the centrifugation suggested that the lack of activity might have been due to loss of a very small quantity of material on the walls of the bowl since the deposit was removed in a cylinder of filter paper placed as a lining around the bowl

Complement Fixation between Antigens made from Normal and Abnormal Livers and Sera from Cases of Homologous Serum Hepatitis Infective Hepatitis and Arsenotherapy Hepatitis

As well as the specific fixation between livers and sera from cases of arsenotherapy hepatitis a reaction was observed occurring more quickly after the onset of symptoms when sera from certain cases of all the three types of hepatitis noted above and some controls were tested against extracts both of normal livers and of those from cases of acute necrosis of the liver This reaction was nearly always more marked in the first specimen of serum obtained than in later specimens and unlike the reaction found in arsenotherapy hepatitis was never seen in specimens of serum taken after the recovery of the patient Complete fixation with serum at a dilution of 1 5 or higher was found in 9 of 84 cases of infective hepatitis (10 7 per cent) in 2 of

this reaction was found in a similar proportion of cases while with sera

This antigen was not destroyed by heating to 56° C for one hour but if it was centrifuged at 2 000 r p m for 10 minutes immediately after heating the antigenic activity was found only in the deposit If left overnight before spinning there was no difference in titre between the supernatant of heated antigen and unheated antigen

This reaction appears to be the same as that described in detail by Eaton *et al* (1944) and similar to the precipitin reaction described by Olitski and Bernkopf (1945)

Heterologous Agglutinins

In the tests for heterologous agglutinins for sheep s erythrocytes a one per cent suspension of erythrocytes was used mixed with an equal volume of each serum dilution The mixtures were left for 15 minutes on the bench spun for from 5 to 10 minutes at 2 000 r p m the sediment shaken up and any agglutination visible to a hand lens 15 minutes after centrifugation

was regarded as positive The recorded titres are final dilutions of serum Absorption tests with both guinea-pig kidney and autoclaved ox erythrocytes were performed by the technique described by Barrett (1941)

RESULTS

The results of the tests for heterologous agglutinins against sheep's erythrocytes are recorded in Tables 50 and 51

TABLE 50

Titre of Agglutinins against One Per Cent Washed Sheep's Erythrocytes

Sera tested	Number of sera giving titre of						Total no of sera tested
	<1/20	1/20	1/40	1/80	1/160	1/320	
From normal people	14	4	1	1	0	0	20
From cases of infective hepatitis	36	2	1	1	1	0	41
From cases of arsenotherapy	18	2	4	0	0	0	24
Dried icterogenic serum	4	6	5	3	3	1	22
Dried serum, not icterogenic	6	4	5	1	3	0	19
Fresh icterogenic serum	12	3	4	0	0	0	19

TABLE 51

Titres for Heterologous Agglutinins (One Per Cent Sheep's Erythrocytes) of Serum from Cases of Homologous Serum Hepatitis and from Cases who Received Dried Serum from Batches Not Known To Be Icterogenic

Cases of homologous serum hepatitis	Titre before absorption	Titre after absorption by guinea-pig kidney	Titre after absorption by 0·2 per cent ox erythrocytes
Me	1 160	1 20	1 160
Dy	1 160	1 20	1 20
Ba	1 160	1 10	1 320
Be	1 320	1 20	0
Br	1 80	0	Not tested
Th	1 80	0	Not tested
Br	1 320	1 40	Not tested
Cases transfused with dried serum (No jaundice)			
Co	1 160	1 10	1 40
Fo	1 160	0	1 40
In	1 80	0	1 80
Su	1 40	1 5	Not tested

No difference from normal was found in sera from cases of infective hepatitis or arsenotherapy hepatitis or from volunteers who had received fresh icterogenic serum. On the other hand cases who developed hepatitis after transfusion or subcutaneous injection with dried reconstituted serum from batches which proved icterogenic, showed the presence of agglutinins much more commonly and often to a high titre Further, agglutinins developed equally in those who showed hepatitis and in those who did not

Investigation of a small series of patients who had been transfused with reconstituted dried serum or plasma not known to be icterogenic and who

had not developed jaundice showed a very similar agglutinin distribution to that of the cases receiving serum from an icterogenic batch

Table 51 shows the results of absorption of the sera from some of the higher titred homologous serum hepatitis cases and from those who had been transfused with reconstituted dried serum of batches not known to be icterogenic by guinea pig kidney and autoclaved ox erythrocytes In all cases there was a very marked fall in the titre of agglutinins after absorption by guinea pig kidney Usually where the titre was 1 40 or below before absorption the titre after absorption was less than 1 5 Absorption by autoclaved ox erythrocytes gave less regular results but in most cases there was some fall in titre not usually so great as after absorption by guinea pig kidney

The rate of development of agglutinins after homologous serum appeared much slower than after the administration of heterologous serum after homologous serum in at least two cases agglutinins were not present one month after transfusion but had reached a titre of 1 80 when tested two months later Unfortunately only a very few sera from cases shortly after transfusion have been available for testing

Discussion

Complement Fixation

The investigation of the complement fixation between liver extracts and sera from cases of infective homologous serum and arsenotherapy hepatitis has revealed two entirely separate and definite reactions Olitski and Bernkopf (1945) brought evidence to suggest that the one is the production of an anti liver antibody occurring in some subjects whose liver has been damaged from any cause Eaton *et al* (1944) who have described the same reaction found it to occur in a larger percentage of cases of all three types of hepatitis than we did as well as in normal subjects Had the criteria of fixation which they accepted been used in our work the number of positives in our studies would have been increased but the close relation to liver damage would have been reduced

The other clear cut reaction is different both in occurring in convalescence instead of in the acute phase of the disease and in being found only between antigens made from the livers of cases of arsenotherapy hepatitis and sera from convalescent cases of the same disease It was at first thought that this reaction was probably due to a combination of arsenic with damaged liver acting as an antigen and numerous experiments were made in an attempt to establish this theory Arsenic could not be demonstrated in the antigen sera containing anti arsenoxide antibodies failed to react with positive antigens inhibition experiments using N A B and mapharside as inhibitors failed in any dilution in which the inhibitors alone were not anti complementary and none of many artificial antigens containing in one case N A B and in the others arsenoxide was effective Therefore this theory must be regarded as unproven

which has now disappeared or become relatively rare This would fit in with Dr Markham's interpretation of the centrifugation experiment, which could not be repeated because antigens of high titre were not again obtained in sufficiently large a quantity Further, Beattie and Marshall (1944) have given evidence suggesting that there is no cross immunity between infective hepatitis and arsenotherapy hepatitis and various authors (p 45, Beeson, Chesney and McFarlan, 1944, Havens, 1945, MacCallum and Bauer, 1944, MacCallum, 1945, Paul Havens, Sabin and Philip, 1945, and Gauld, 1946) have failed to find evidence of cross immunity between infective hepatitis and homologous serum hepatitis although there is clear-cut evidence that either an attack of infective hepatitis or a subclinical infection does give rise to some immunity (Gauld, 1946) Oliphant (1944) however failed to produce hepatitis in ten volunteers who had previously suffered from homologous serum hepatitis, and were given infective hepatitis material which caused jaundice in four out of ten normal volunteers Most of the evidence suggests a different causation for infective hepatitis from that for homologous serum hepatitis or arsenotherapy hepatitis, but there is no evidence for or against cross-immunity between the latter two conditions

Some cases of hepatitis during arsenotherapy continued to occur after the alteration of technique which eliminated the likelihood of syringe spread This fact, combined with the failure of the complement fixation reaction in ten successive cases, seven of them tested at all stages of the disease, suggests multiple causation Such an explanation might account for the high percentage both of convalescent sera and of livers from fatal cases which failed to show activity even during the epidemic period

Findlay, Martin and Mitchell (1944) report obtaining an antigen by saline extraction of the liver of a fatal case of hepatitis in an African soldier This antigen gave complement fixation with sera from cases of post-vaccinal and infective hepatitis, but not with control sera The positive sera gave negative fixation with an extract of normal liver Four other livers were tested, two of them gave some fixation and two others none at all In the series reported here the liver extract from one case of infective hepatitis gave suggestive results with sera from the same disease, but not with sera from cases of arsenotherapy hepatitis The remainder of the large series of livers from known or reputed cases of infective hepatitis or homologous serum hepatitis gave negative results

Heterologous Agglutinins

The presence of increased titres of heterologous agglutinins for sheep's erythrocytes in the cases with homologous serum hepatitis is in line with the finding by Eaton *et al* (1944) of higher titres in post vaccinal than in infective hepatitis It has not, however, proved possible to confirm their finding of an increased titre in infective hepatitis. Bercovitz and Knoch (1944) and Barker *et al* (1945) have also reported a normal distribution of heterologous agglutinins in cases of infective hepatitis

The evidence presented above suggests that the raised titre in homologous serum hepatitis is due to transfusion with dried reconstituted serum or plasma rather than to hepatitis Even where there are raised heterologous agglutinins in homologous serum hepatitis, absorption by guinea pig kidney and autoclaved ox erythrocytes will serve to differentiate them from those of infectious mononucleosis

juice collected from cases of infective hepatitis on their first day of jaundice Two pigs received duodenal juice orally and blood intraperitoneally No faeces were used for inoculation, and no passages were performed Estimations of the pigs' serum bilirubin were made at frequent intervals The animals were killed from 5 to 60 days later, and there was no discernible macroscopic or microscopic abnormality

GOLDEN HAMSTERS

Fifty normal golden hamsters (*Cricetus auratus*) were used Five different series were initiated, and duodenal juice, blood and urine collected from cases of infective hepatitis on their first day of jaundice or earlier were administered orally and intraperitoneally One to five blind passages* were made with suspensions of liver, spleen, kidney and brain of the inoculated animals, at 14 to 21-day intervals The hamsters' temperatures were taken and their urine was examined for bile pigment The animals remained well, when killed and examined 14 to 96 days after inoculation, no macroscopic or microscopic abnormality was detected

ORKNEY VOLES

Inoculation of human material, followed by blind passages at 14- and 21 day intervals, in 29 Orkney voles (*Microtus orcadensis*) also yielded negative results In some of these animals direct inoculation into the liver and spleen was performed

COTTON RATS

One hundred cotton rats (*Sigmodon hispidus hispidus*) from Eastern U S A were used in four series of blind passages initiated by inoculation of blood, duodenal juice, faeces and urine from cases of infective hepatitis at the time of their first appearance of jaundice or earlier (The animals actually used in the experiments were bred in England) Two to fourteen blind passages of organs were carried out in 10- to 21 day old animals In nearly every instance both initiating and passage inoculations were given by the oral, intraperitoneal and intrahepatic routes, and sometimes intracerebrally as well Temperatures were taken daily in several experiments, and blood and urine were examined There were no detectable clinical abnormalities and no histological changes other than the presence of type B inclusions in the nuclei of numerous tubule cells of the kidneys of animals of the 7th to 12th passages in one series These inclusion bodies were not seen in animals of the 13th and 14th passages in this series and only rarely in uninoculated animals Subcutaneous inoculation of a bacteriologically sterile suspension of liver spleen and kidney from animals of the 10th, 11th and 12th passages of the series into 10 human volunteers did not produce disease

GUINEA-PIGS

It has been shown that the guinea pig foetus can readily be infected *in utero* with certain viruses to which the adult guinea pig is only slightly susceptible There is also the possibility that the metabolic state of the pregnant guinea pig may render it more susceptible to infection For these reasons attempts were made to transmit infective hepatitis to pregnant guinea

* *Blind passage* is the inoculation of the suspensions of the tissues of animals showing no symptoms or signs of disease

pigs and their foetuses Thirty pregnant guinea pigs were inoculated with negative results

Several series of one to five passages in 38 guinea pigs 5 to 14 days old, also failed to produce any clinical or histological abnormalities Verlinde (1944) in Leyden reported successful transmission of infective hepatitis to guinea pigs by inoculation of human blood and urine from cases in the early stages of the disease We failed to produce any evidence of disease in our guinea pigs when inoculated with dried guinea pig tissues sent to us by Verlinde

CANARIES

Twenty canaries that had survived drug tests in malarial studies were inoculated with duodenal juice urine and serum by the method described by Dresel Meding and Weineck (1943) but neither the illness with a short incubation period described by them nor the disease with a long incubation period described by Herzberg (1943) was observed

MICE

As innumerable previous attempts to infect mice with material from cases of infective hepatitis had been unsuccessful the only experiment in which mice were used was a series of passages following inoculation with liver biopsy material obtained from cases of infective hepatitis on their first day of jaundice This method was claimed by Dohmen (1943) to have produced positive results Five passages of organs at 14 and 21-day intervals in 27 mice failed to produce any significant histological changes in mice killed after intervals of 14 to 50 days

Though little has been written on the subject the presence of necrotic lesions and inclusion bodies in the livers of many apparently normal stock mice has been recognized for some time (Findlay 1932) Recently Nicolau and Ruge (1944) in Roumania and Olitski and Casals (1945) in the United States have described such findings in their stock mice while studying infective hepatitis In addition Nicolau and Ruge have also described what they considered were specific intranuclear inclusions and elementary bodies in the tissues of mice guinea pigs and rabbits inoculated with infective hepatitis material The animals showed no symptoms and the condition could not be passed in series It must be concluded therefore that the mouse is an unsatisfactory animal to use for studying infective hepatitis

RATS

Attempts were made to lower the resistance of white rats of the Wistar strain of varying ages by exposure to 250 roentgens (12 rats), by splenectomy and treatment with neoarsphenamine to cure any resulting bartonellosis (12 rats), by bi weekly intraperitoneal injections of 14 times the human dose of neoarsphenamine and "mapharsen" for from 2 to 8 weeks (19 rats) and by infection with *S typhi murium* (28 rats) All these procedures were unsuccessful in rendering the animals susceptible to the virus of infective hepatitis when they were fed with duodenal juice or injected with blood intraperitoneally from early cases of infective hepatitis

In view of the numerous reports of the injurious effect of a low protein diet on the liver of the rat (Himsworth and Glynn 1944), experiments were made on rats fed on a diet which when used by Himsworth and Glynn,

had produced minimal microscopic necrotic lesions and those only occasionally It was hoped that the diet might render the rats susceptible to the virus of infective hepatitis The results of some of these experiments have already been described in a preliminary communication (MacCallum and Miles, 1946) The first series, in which blood, faeces and urine from several cases of *infective hepatitis** were used for inoculation, was carried through five passages, a total of 39 rats being employed Only a very occasional microscopic collection of round cells or an occasional abnormal parenchymal cell was seen in the livers of these rats, and neither subcutaneous nor oral inoculation of liver from 4th passage rats into 10 human volunteers produced disease

A second series gave more promising results Six rats were given 8 g daily of a diet containing protein in the form of casein (4 per cent by weight of total diet) and dried yeast (16 per cent by weight of total diet, i e approximately 7 2 per cent protein), for a preliminary period of 40 days prior to inoculation with faeces, urine and blood from patients in the early stages of infective hepatitis Blind passages with organ suspensions were then carried out at intervals of 16 to 21 days in 130 rats which had had a preparatory period of 50 to 92 days on the deficient diet Microscopic lesions in parenchymal liver cells were found in a rat of the 3rd passage, and macroscopic necrosis was present in the livers of 2 rats of the 4th passage For the subsequent passages the preparatory period on the deficient diet was 7 to 21 days Haemorrhages in the lungs, stomach, intestines and lymph glands and an inflammatory reaction in the spleen appeared in the 5th and subsequent passages There was considerable variation in the degree of the pathological changes in different animals of the same passage, some showing numerous macroscopic lesions at death, others remaining apparently well but showing microscopic lesions if killed within 60 days of inoculation There appeared to be an increase of virulence with passage up to a certain stage, and the infectivity of the agent could be reduced by dilution

The lesions failed to appear after the 12th passage All attempts to induce the same pathological picture with organs from previous passages that had been stored at temperatures of −20°C or −70°C were unsuccessful Three further attempts to reproduce the disease under the same conditions in Wistar strain rats by inoculation of fresh blood and faeces collected during the acute stage of the disease from typical cases of infective hepatitis were also unsuccessful

The incubation period to onset of illness was usually about 18 days from the day of inoculation, though some rats died with acute necrosis of the liver 21 days after starting on the diet and 14 days after injection Some

intervals failed to increase the virulence of the agent or reduce the average incubation period of the disease

Rats which had been on a similar preparatory diet in which, however, the casein had been increased to 6 per cent were inoculated with material from the 7th passage of the above series The macroscopic lesions in the

* These cases were from a large outbreak of infective hepatitis in an institution (p 37)

lymph glands and stomach and microscopic changes in the liver and spleen were present at this first passage and were produced as frequently, and after the same intervals, as in the rats on the diet containing 4 per cent. of casein Macroscopic necrosis of the liver, however, was not seen till the 4th passage No macroscopic or microscopic lesions appeared in rats on a diet containing 8 per cent casein and 16 per cent dried yeast

Five rapid blind passages were carried out at 10 to 11-day intervals in recently weaned, Wistar strain rats fed on a diet of bread and milk and "marmite", but no macroscopic or microscopic lesions were produced Attempts to reproduce the condition by inoculation of rat tissues into 6-week old mice of several strains on a normal diet were also unsuccessful, even after five blind passages of tissues in 30 mice

Though we, like others, had made numerous unsuccessful attempts during several years to infect guinea pigs with human material from cases of infective hepatitis, the recent report of transmission to this animal by Verlinde encouraged us to try to infect them with the presumed rat virus Five very young guinea pigs were inoculated orally and intraperitoneally with a 750 mμ membrane filtrate of a suspension of tissues from the 9th passage of the rat series After 33 days 2 animals were killed in spite of having shown no signs of illness, and their livers, spleens and kidneys were removed A suspension of these tissues was inoculated into 4 more very young guinea-pigs These were dealt with in the same manner Two more similar passages were made, during which all the animals remained well At the 3rd passage all 4 animals were moribund or dead on the 8th day with acute haemorrhagic necrosis of the liver and severe fatty degeneration Further passages with these tissues failed to produce any lesions nor could the lesions be produced by inoculation of another sample of the material used in the 3rd passage, even when the animals were put on a quantitatively reduced diet.

Verlinde later informed us that a dried suspension of liver and spleen from our 13th rat passage series produced slight lesions of the liver in his strain of guinea pigs We had found no histological lesions in the rat tissues sent to him for these inoculations

After two passages of rat tissues inoculated into the allantoic and yolk sacs of 7-day old chick embryos, pools of embryos and their membranes failed to produce lesions when administered orally and intraperitoneally to rats on the 4 per cent casein diet

Two human volunteers received 35 ml of a 760 mμ membrane filtrate of the rat tissues by mouth After 6 months neither had shown any signs or symptoms suggesting infection

A neutralization test using the convalescent serum of the patients supplying the original material, though not entirely satisfactory gave suggestive results Owing to a shortage of rats and to loss of the hypothetical agent it was not possible to obtain further information on this point.

In routine passages and titration experiments all material was passed through a gradocol membrane filter with an average pore diameter of 750-760 mμ In one experiment typical lesions were produced with a filtrate which had passed a 310 mμ membrane, the smallest size used in the experiment. typical lesions were also produced in another experiment in which 310 mμ, 113 mμ, and 63 mμ membranes were used Further experiments to

*

determine the size of the agent were not carried out before passage became unsuccessful

In control experiments, rats of the same strain and weight, and on the same 4 per cent. casein diet as those in which lesions had been produced, were inoculated with serum and faeces from healthy subjects, and blind passages were carried out at 19 to 21-day intervals for nine passages At the time of each passage only a proportion of the animals was killed, the remainder being examined for histological changes at later dates, up to 70 days No lesions were observed in any of this series In addition no lesions were seen when our rats were inoculated with material from rat livers rendered necrotic as a result of a protein deficient diet only These inoculations were carried out in 1943 using necrotic rat livers provided by Himsworth and Glynn and were to serve as a control to their dietary experiments

These experiments suggest that we isolated a virus which produced a disease in rats Proof that it was a causative agent of infective hepatitis was not obtained There was also the possibility that we were dealing with a virus normally latent in the rat which had been activated by the dietary deficiency and serial passage As a control to the experiments of Himsworth and Glynn and our own a further series of controls was tested Rats on the 4 per cent casein diet in our laboratory were injected with necrotic livers produced by a protein-deficient diet. Seven passages failed to produce any significant lesions

We are indebted to Dr F Bergl of Roche Products for the generous supply of vitamins for the rat diets, and to Dr F G Spear of the Strangeways Research Laboratory, Cambridge, for irradiation of the rats, and to Dr H. P. Himsworth for frequent help and advice The experiments on pigs were made by permission of Dr C. A. McGaughey of the Institute of Animal Pathology, Cambridge The inoculations and bleedings were performed by Mr J. A. J Venn, B V Sc

have shown that in the early stages of the disease duodenal juice and blood contain the infective agent By the oral administration of a large quantity of urine from a case of infective hepatitis, Voegt has also produced what appeared to be a mild hepatitis without jaundice in a volunteer

It was decided, therefore, that experiments on human volunteers were essential if further knowledge was to be obtained on the mode of spread and duration of infectivity of the various types of hepatitis designated as infective, homologous serum and arsenotherapy hepatitis

The first volunteers were conscientious objectors, they provided, however, only a limited number of subjects and they mostly participated in the experiments by MacCallum and Bauer (1944) on homologous serum hepatitis. In view of the clinical observation by Still (1897) and Hench (1938) that patients suffering from rheumatoid arthritis underwent remissions of their illness during the course of attacks of jaundice of unspecified cause, and that injections of large quantities of bilirubin had no beneficial effect on them (Hench, 1938), it was considered justifiable to invite such patients to volunteer for inoculation with material from cases of the types of hepatitis under study With the co-operation of their own physicians patients were inoculated at various centres, the majority of the inoculations being done in Oxford and Sheffield The effects of the jaundice on the arthritis have already been reported (Gardner, Stewart and MacCallum, 1945), and will not be discussed here

INFECTIVE HEPATITIS

The majority of doctors who witnessed the epidemics of infective hepatitis in troops in the Middle East in 1914 18 and in 1942-43 were of the opinion that the source of spread was probably infected faeces This conception was dependent upon the high incidence of associated dysentery and the prevalence of flies (Willcox, 1919, Spooner, 1944 Kirk, 1945)* In Sweden, Hallgren (1943) had suggested that waterborne infection was the cause of an institutional epidemic and of scattered cases along a river

In Great Britain, on the other hand, the epidemiological studies of Pickles (1939) and others (see also p 46) have suggested that infection is due to the airborne spread of particles from the nasopharynx Because of these latter views and a certain reluctance to administer faecal material to man, it was decided to do experiments successively with nasopharyngeal washings, blood, urine and faeces, in that order The methods of preparation, transport and administration of material are described below

Although obviously desirable, it was impossible to carry out the experiments in an isolated community, and this possibly lays open to criticism the conclusions drawn from certain experiments As small attack rates were usually to be expected, large groups of individuals were used

It was considered likely that the highest concentration of the infective agent in the blood and nasopharynx would be found during the pre icteric stage of the disease A similar assumption was not made in the case of the faeces and urine, but whenever possible all specimens were collected in the pre icteric and early icteric phases. Owing to lack of epidemics in large closed communities and to the difficulty of diagnosing the disease in its early

* Van Rooyen planned transmission experiments in human volunteers with intestinal contents and urine in the Middle East in 1943 but permission to use human volunteers was not granted (van Rooyen and Kirk 1946)

determine the size of the agent were not carried out before passage became unsuccessful

In control experiments, rats of the same strain and weight, and on the same 4 per cent casein diet as those in which lesions had been produced, were inoculated with serum and faeces from healthy subjects, and blind passages were carried out at 19- to 21-day intervals for nine passages At the time of each passage only a proportion of the animals was killed, the remainder being examined for histological changes at later dates, up to 70 days No lesions were observed in any of this series In addition no lesions were seen when our rats were inoculated with material from rat livers rendered necrotic as a result of a protein-deficient diet only These inoculations were carried out in 1943 using necrotic rat livers provided by Himsworth and Glynn and were to serve as a control to their dietary experiments

These experiments suggest that we isolated a virus which produced a disease in rats Proof that it was a causative agent of infective hepatitis was not obtained There was also the possibility that we were dealing with a virus normally latent in the rat which had been activated by the dietary deficiency and serial passage As a control to the experiments of Himsworth and Glynn and our own, a further series of controls was tested Rats on the 4 per cent casein diet in our laboratory were injected with necrotic livers produced by a protein-deficient diet Seven passages failed to produce any significant lesions

We are indebted to Dr F Bergl of Roche Products for the generous supply of vitamins for the rat diets, and to Dr F G Spear of the Strangeways Research Laboratory, Cambridge, for irradiation of the rats, and to Dr H P Himsworth for frequent help and advice The experiments on pigs were made by permission of Dr C A McGaughey of the Institute of Animal Pathology, Cambridge The inoculations and bleedings were performed by Mr J A J Venn B V Sc

Transmission Experiments in Man

by

F O MacCallum, M D, Alice Stewart, M D, F R C P, and W H Bradley, D M,

in collaboration with

D J Bauer, M B, Ph D, H L Milles, M C, M R C P, J B Rennie, M D, M R C P, J G Sclater, M B, F R C P E, Sheila Sherlock, M D, M R C P, and E G L Bywaters, M B, M R C P

The failure to produce a disease in experimental animals by inoculation of blood and excreta from cases of infective hepatitis has prompted workers in several countries to carry out experiments in human volunteers The experiments of Voegt (1942) in Germany and of Cameron (1943) in Palestine

have shown that in the early stages of the disease duodenal juice and blood contain the infective agent By the oral administration of a large quantity of urine from a case of infective hepatitis, Voegt has also produced what appeared to be a mild hepatitis without jaundice in a volunteer

It was decided, therefore, that experiments on human volunteers were essential if further knowledge was to be obtained on the mode of spread and duration of infectivity of the various types of hepatitis designated as infective, homologous serum and arsenotherapy hepatitis

The first volunteers were conscientious objectors, they provided, however, only a limited number of subjects and they mostly participated in the experiments by MacCallum and Bauer (1944) on homologous serum hepatitis In view of the clinical observation by Still (1897) and Hench (1938) that patients suffering from rheumatoid arthritis underwent remissions of their illness during the course of attacks of jaundice of unspecified cause, and that injections of large quantities of bilirubin had no beneficial effect on them (Hench 1938), it was considered justifiable to invite such patients to volunteer for inoculation with material from cases of the types of hepatitis under study With the co operation of their own physicians patients were inoculated at various centres, the majority of the inoculations being done in Oxford and Sheffield The effects of the jaundice on the arthritis have already been reported (Gardner, Stewart and MacCallum 1945), and will not be discussed here

INFECTIVE HEPATITIS

The majority of doctors who witnessed the epidemics of infective hepatitis in troops in the Middle East in 1914 18 and in 1942 43 were of the opinion that the source of spread was probably infected faeces This conception was dependent upon the high incidence of associated dysentery and the prevalence of flies (Willcox, 1919, Spooner, 1944, Kirk 1945)* In Sweden, Hallgren (1943) had suggested that waterborne infection was the cause of an institutional epidemic and of scattered cases along a river

In Great Britain, on the other hand, the epidemiological studies of Pickles (1939) and others (see also p 46) have suggested that infection is due to the airborne spread of particles from the nasopharynx Because of these latter views and a certain reluctance to administer faecal material to man it was decided to do experiments successively with nasopharyngeal washings blood, urine and faeces, in that order The methods of preparation transport and administration of material are described below

Although obviously desirable, it was impossible to carry out the experiments in an isolated community, and this possibly lays open to criticism the conclusions drawn from certain experiments As small attack rates were usually to be expected, large groups of individuals were used

It was considered likely that the highest concentration of the infective agent in the blood and nasopharynx would be found during the pre icteric stage of the disease A similar assumption was not made in the case of the faeces and urine but whenever possible all specimens were collected in the pre icteric and early icteric phases Owing to lack of epidemics in large closed communities and to the difficulty of diagnosing the disease in its early

* Van Rooyen planned transmission experiments in human volunteers with intestinal contents and urine in the Middle East in 1943 but permission to use human volunteers was not granted (van Rooyen and Kirk 1946)

stages, much of the material from cases early in the disease was derived from the patients studied by Wilson who were admitted to hospital on the day of onset of clinical jaundice or later (see p 50)

Methods

The various materials used in these experiments were collected, stored and administered in the following ways —

Nasopharyngeal washings There were slight variations in the methods used to obtain washings, depending on the age and clinical condition of the donor Usually 10 ml of saline or broth were instilled into the nose with a pipette, allowed to drain into the nasopharynx and then expectorated into a clean bowl Without delay these washings were transferred to a sterile flask or bottle standing in ice The walls of the buccal cavity were then syringed with a further 10 ml of broth or saline, the patient gargled with this fluid and it was then added to the first specimen In some instances larger amounts than 20 ml were collected from donors In one large experiment in which the donors were naturally-occurring cases of infective hepatitis and in another in which the material was collected from cases of homologous serum hepatitis, the tonsils were thoroughly rubbed with throat swabs which were then stirred into the pooled washings already obtained Where delay between collection and use of the specimens was inevitable, they were transported to the laboratory in a thermos flask containing ice Here they were stored at $-20°C$ till the day of use when they were transported in bottles packed in ice to the place of inoculation When direct transmission was performed the receptacle containing the washings was kept in ice for a period of up to one hour before being administered to the recipients The recipients received the material either lying down with the head tilted well back over a pillow, or seated in a chair with the head tilted back The washings were in every case sprayed into the nose by an atomizer or dropped in with a pipette, then sprayed on to the pharynx and walls of the buccal cavity with an atomizer or syringe and finally the fluid was swallowed When large amounts of washings were available, 10 ml amounts were also gargled and then swallowed When swabs from the donors' tonsils had been obtained the tonsillar areas of the recipients were also firmly rubbed with the swabs which had been soaked in the pooled washings

Blood All specimens of blood used were obtained by venepuncture and placed in sterile containers In most cases the serum was separated and removed within a few hours, but in some instances separation was not carried out for 24 to 48 hours and then sometimes the serum contained a small number of red blood cells In only one instance were both serum and clot injected After separation, seven samples of serum were stored for from 3 to 14 days at $-20°C$ before use, when they were transported packed on ice to the place of inoculation A single specimen, No 10 in Table 53 (p 122) was stored for 4 months at $-20°C$ before use One lot of pooled serum No 11, was injected into the first three recipients within 3 hours of collection and then stored at $+4°C$ for 1 to 3 days before being given to the remaining three recipients A single specimen, No 12, was frozen and dried *in vacuo*, and then stored for 6 months at $+2°C$ before use In ten experiments, the serum was injected subcutaneously in 0.5 to 1.0 ml amounts In Experiment No 11 (b) the clot was ground in a mortar and suspended in

10 ml saline, 1 0 ml of the suspension was sprayed intranasally, and 1 0 ml of the serum was sprayed on to the oropharynx with a syringe and needle and then swallowed

Urine All the specimens of urine used were from males, and were collected in sterile bottles, after thorough cleaning of the external meatus They were either admission or early morning specimens, and after cultures had been made from them they were stored at -20°C till use All specimens used were collected within 24 hours of the appearance of scleral icterus, gave positive iodine tests, and were brown in colour, so that considerable amounts of bilirubin were present In the single experiment the six specimens were pooled, and each recipient received 2 0 ml sprayed into the nostrils, 2 0 ml sprayed on to the oropharynx and swallowed, and 5 0 ml to drink

Faeces Faecal specimens were collected in clean bottles and stored at -20°C When required, weighed amounts were ground in a mortar with saline or broth and made up as 10 per cent suspensions These were lightly centrifuged for 15 minutes, the supernatant removed, and to it 10 per cent by volume of ether added The supernatant was then placed in a flask with a rubber stopper in the refrigerator and shaken at frequent intervals After 24 hours the ether was evaporated and the faecal suspension stored at -20°C In preparing the filtrate for Experiment No 20 (Table 55, p 125), the faecal suspension was not treated with ether but diluted to 3 per cent with broth, spun at 3,000 r p m for 30 minutes and the supernatant passed through a gradocol membrane filter with an average pore diameter of 730 mμ Part of the filtrate was stored at -20°C, and part frozen and dried *in vacuo* in 1 0 ml amounts and stored at 0°C till used Immediately before nasal and oral administration, a small crystal of vanillin was added to all faecal suspensions The suspensions were sprayed into the nostril and on to the oropharynx, and 5 to 10 0 ml amounts were given to drink mixed with orangeade In Experiment No 21 the dried material was redissolved in 1 0 ml of sterile distilled water and injected subcutaneously

Results

Because of the widespread shortage of staff, both in hospitals and laboratories it was usually not possible to perform detailed liver function tests, such as the bromsulphalein excretion test or the hippuric acid synthesis test The routine tests employed were either the iodine test or Hunter's test for bilirubin in the urine, and the measurement of the serum bilirubin by Thannhauser and Andersen's modification of the Van den Bergh reaction

As a rule patients were seen once a week, but they reported on other days if they had any suggestive symptoms They were then examined daily until the symptoms disappeared and the laboratory tests showed no abnormality As beds were scarce, patients who developed jaundice were hospitalized only if the illness was severe Out of the two hundred persons inoculated with infective hepatitis material, only twelve developed hepatitis with jaundice, and another five had what was possibly a very mild hepatitis As these latter five cases occurred among large groups of recipients in whom there were no cases of frank jaundice or undoubted hepatitis, they are only referred to in this report as " possible takes " and not as true transmissions, although two of them have been described as " positives " in a previous preliminary communication

(MacCallum and Bradley, 1944) The experiments described as Nos 1, 2 and 3 (Table 52), No 13 (Table 54) Nos 8 and 9 (Table 53), and No 18 (Table 55) have all been referred to in this earlier preliminary communication.

Nasopharyngeal washings All our attempts to produce jaundice by inoculation of nasopharyngeal washings, garglings and tonsillar swabbings were *unsuccessful (Table 52)* Two of the patients in the first group of seven recipients (Experiment No 1) showed evidence suggestive of hepatitis, but they did not become jaundiced

[illegible]

he is not now regarded as a "take" with the nasopharyngeal washings

The second "possible take" in this group was a man aged 45 who suffered from rheumatoid arthritis and had a chronically enlarged palpable but not tender liver [illegible]

arthritis

These two cases are now no longer regarded as "takes"

The material used in Experiment No 1 had been stored 7 to 16 days at −20°C. before use Similar material from two other groups of donors was negative (Experiments Nos 2 and 3) It was then obvious that *direct* transmission experiments must be done with material collected at the onset of the illness before accepting the failure of inoculation with nasopharyngeal washings It was decided, therefore, to induce jaundice in a group of *volunteers by the administration of faeces from cases of infective hepatitis* so as to be able to collect fresh material under the best conditions (The results of experiments in this country (MacCallum and Bradley, 1944) and in the U S A (Havens Ward, Drill and Paul, 1944) had indicated that this procedure would be the most likely to produce cases most rapidly) *Faecal* material was administered to twenty patients (Experiment No 19 (a) Table 55) and, though none of them showed any symptoms, nasopharyngeal washings and garglings were collected on the 21st, 23rd and 25th days and administered to eighteen recipients within an hour The pool failed to produce hepatitis in any of the recipients although parts of previously positive pools, from Experiments Nos 14-17, were included in the fresh material and of course none of the recipients of the nasopharyngeal washings developed hepatitis

A small epidemic of jaundice occurred in a school situated close to the clinic where some of the volunteers with arthritis were being treated Three *weeks* after the first case in the school, we visited the classrooms looking for children showing possible early symptoms Three sick children were found, but unfortunately none of them ever became jaundiced though they had such positive signs as bilirubin in the urine and serum bilirubin rising to 2 0 to 2 25 mg per 100 ml There were several cases of jaundice in the same

TABLE 52

Human Transmission Experiments with Nasopharyngeal Washings from Cases of Infective Hepatitis

Experiment No	*No of donors in pool*	*Time of collection of nasopharyngeal washings*	*Route of administration*	*Amount (ml)*	*No of recipients*	*No of recipients becoming jaundiced*	*Incubation period (days)*
1	6	All on day of onset of jaundice	Nasal and oral (after storage)	4	7	0	–
2	6	All on day of onset of jaundice	Nasal and oral (after storage)	4	9	0	
3	2	One on 1st day of symptoms one on day of onset of jaundice	Nasal and oral (after storage)	7	6	0	–
4	3	One on 5th day of symptoms and following 2 days one on 3rd day of symptoms and following 2 days one on 1st day of symptoms and following 2 days	Nasal and oral tonsillar swabs	20–30	18	0	

classes in this school before and after the donors were used and the cousin of one of them had also had jaundice 3 weeks previously Though these experiments gave negative results (see Experiment No 4 in Table 52) they will be described in detail for the donors are good examples of the type of cases that have so often been suspected as being the source of infection in epidemics where there have been long intervals between cases of frank jaundice

symptom free

Four days later two ill girls were found in another class where there had been a case of infective hepatitis several weeks previously One had nausea and vomiting for the past 2 days and was now feeling better but Hunter's test for bilirubin in the urine was strongly positive The other girl's symptoms had begun with vomiting

The two girls were also bled on the 2 days when the large scale naso pharyngeal washing transmission experiment (No 4) was carried out but

as will be seen from Table 53. Experiments Nos 11 (a) and (b) none of the recipients receiving this blood either by parenteral or oral routes showed any symptoms of hepatitis

The recipients in Experiment No 4 were all between the ages of 32 and 63 with only seven under 40, and their susceptibility may well have been diminished by their age On the other hand there may have been little or no virus present in the nasopharynx and oropharynx of any of the donor children at the time of collection of the washings

TABLE 53

Human Transmission Experiments with Blood from Cases of Infective Hepatitis

Experiment No	No of donors in pool	Time of collection of blood	Route of administration	Amount (ml)	No of recipients	No of recipients becoming jaundiced	Incubation period (days)
5(a)	1	Day of onset of jaundice	Parenteral	0 5	5	1	27
(b)	1	2nd day of jaundice (same man)	Parenteral	0 5	4	1	49
6(a)	1	Day of onset of jaundice	Parenteral	0 5	6	0	-
(b)	1	6th day of jaundice (same man)	Parenteral	0 5	6	0	-
7	1	Day of onset of jaundice	Parenteral	0 5	4	2	34 36
8	2	Day of onset of jaundice	Parenteral	0 5	4	3	64 75 92
9	2	Day of onset of jaundice	Parenteral	0 5	2	0	-
10	1	Sub-icteric serum bilirubin 0 25 mg /100 ml	Parenteral	0 5	4	2	63, 69
11(a)	2	Sub-icteric 1st day of symptoms in one 3rd in the other	Parenteral	0 5	6	0	-
(b)	2	Same serum and clot	Nasal and oral	2	10	0	-
12	1	Day of onset of jaundice, dried *in vacuo* 6 months	Parenteral	1	4	0	-

Blood It has already been shown by Voegt (1942) and Cameron (1943) that whole blood and serum collected at the time when jaundice appears may be infective But the finding of virus in the blood gives little information regarding the natural mode of transmission of infective hepatitis or of the duration of infectivity The injection of blood from patients may, however, be used to determine the length of the incubation period of the experimentally produced disease

Ten different samples of serum from cases of infective hepatitis were tested, with the exception of three, each of these samples was from a single donor It was found that the incubation period from the parenteral inoculation of serum to the appearance of jaundice was less than 40 days in three patients, and 49 to 92 days in the remaining six positive "takes" The three patients who had the short incubation periods were all about 30 years of age One of these three had been the first doubtful positive with the nasal washings (see p 120), and one had had a previous attack of hepatitis

following an injection of serum from an icterogenic pool of dried transfusion serum The man with the incubation period of 49 days (Experiment 5 (b)) was 30 years of age, he had had a mild attack of homologous serum hepatitis following an injection of icterogenic serum a little more than 6 months previously The three recipients in Experiment No 8 who developed jaundice were cases of rheumatoid arthritis, of 33, 35 and 42 years of age, and the two in Experiment No 10 who developed jaundice were also patients with rheumatoid arthritis and were aged 42 and 50 Serum from one man collected on the day of onset of jaundice failed to induce hepatitis in six recipients aged between 21 and 59 (Experiment No 6 (a)) This man was unfortunately chosen to be tested at weekly intervals to see how long his blood would remain infective However, the original sample and the one collected on the 6th day gave negative results, and so remaining samples, which had been stored, were not tested The completely negative results in Experiment No 11 (a) and (b) are difficult to explain, but the low susceptibility of normal individuals to parenteral injection of infective hepatitis serum has been noted by Neefe and Stokes (1945) The donors were the two girls whose illnesses are described in detail on p 121 In Experiment No 11 (b) the clots from both bloods taken on each of 2 days were ground in a mortar and suspended in 10 ml of saline With each recipient, 1 0 ml of this suspension was sprayed intranasally and 1 0 ml of the serum from the same specimens of blood was sprayed on to the oro-pharynx and swallowed In Experiment No 11 (a) the serum was injected parenterally The age of the recipients may have influenced the result, of the six recipients in the parenteral group there were two from each of the age groups 20 to 30, 40 to 50, 50 to 60, the recipients in the oral group were aged 19, 39, five between 40 and 50, two between 50 and 60 and one was aged 70 Another explanation might be that the strain of virus was of such low virulence that it was ineffective by the oral route in the small dose used The donor in Experiment No 12, which was also negative was a typical case of infective hepatitis from a military unit who had been jaundiced for 24 hours when the blood was collected Three of the four recipients in this experiment developed hepatitis with jaundice when later inoculated with a known batch of icterogenic dried transfusion serum Their ages were 30, 36, and 52

The most interesting result of the experiments with blood was the potency of the serum used in Experiment No 10 (Rennie and Fraser, 1946) This serum was obtained from a mentally defective child in an institution where an epidemic was in progress, and where routine tests were being done on contacts of cases (p 37) The patient had no apparent symptoms and the serum bilirubin of the specimen used was only 0 25 mg per 100 ml as measured by the method of Malloy and Evelyn The patient was never observed to be jaundiced, but his serum bilirubin rose to 2 5 mg per 100 ml 4 days later

Urine A single experiment was carried out using a large pool of bacteriologically sterile urine, collected from six men on the first day of the appearance of jaundice Each recipient received a total of 9 0 ml (Table 54) In addition to the measurement of the serum bilirubin, the liver function of each of the nineteen recipients was tested frequently by the oral hippuric acid synthesis test There were no cases of jaundice among the recipients Three of them showed some dubious evidence of hepatic dysfunction, but they were not

accepted as positive transmissions It is possible that the amount of urine administered was too small to be infective This experiment refers to the same group as has been described by MacCallum and Bradley (1944), and by Rennie and Fraser (1946)

Faeces Successful transmission of infective hepatitis by means of faeces has already been briefly reported in a preliminary communication by MacCallum and Bradley (1944) These findings are here referred to in Table 55 (Experiments Nos 14, 15, 16 and 17) In Experiments Nos 14 and 15 the six recipients in each experiment had 2 0 ml of faecal suspension sprayed by atomizer into the nostrils, and 2 0 ml sprayed on the oropharynx and swallowed A few hours after inoculating these two groups, two further volunteers became available About 2 0 ml each of the suspensions used for Experiments Nos 14 and 15 remained, these were pooled and administered intranasally to the two additional recipients in a dose of 2 0 ml, and both of these latter recipients developed mild illness with jaundice (Experiment No 16) The material used in Experiment No 17 was collected from three Service patients, who were detected in the early stages of infective hepatitis, several days before the appearance of clinical icterus The seven recipients in this experiment all received their material in the same manner as those in Experiments Nos 14 and 15 Only one, a young adult, developed jaundice after 27 days Experiment No 18 was expected to provide a high proportion of "takes" since the material used in it came from two patients with infective hepatitis, one a nurse, one a patient, who were living in an institution where an epidemic was occurring, the virus of which seemed to be more than usually infective (p 37), the specimens were taken on the first day of jaundice in one case and on the day preceding clinical jaundice in the other There was no evidence that any of the five recipients suffered from hepatitis following the inoculation of this material Experiment No 19 (a) also failed to produce any positive results though the inocula used included some of the remnants from Experiments Nos 14, 15, 17 and 18, which had been kept stored for 4 to 7 months at $-20°C$ The case providing material on the 4th day of symptoms was one that had had jaundice induced by injection of serum in Experiment No 8 (Table 53) One week later all the recipients from Experiment No 19 (a) were reinoculated, the donor in this experiment, No 19 (b), was the serum induced case in Experiment No 5 (a) (see Table 53) The small number of positive successes as compared with the high proportion recorded in most experiments by workers in the U S A, may have been due either to attenuation or inactivation of the virus caused by the treatment of the faeces with ether, or to the comparatively greater resistance of the volunteers, perhaps on account of their age (nearly all were over 30)

Discussion

As a result of our experience, which agrees with that of Neefe Stokes and Reinhold (1945) and Havens (1946a) in the United States, it would appear that there is little or no virus present in the nasopharynx and oropharynx of patients with infective hepatitis during the clinical illness Further experiments may show that the virus is present in these sites shortly after contact with an infected patient and that further transmission may take place from these sites at this time But this must be considered as purely hypothetical until definite evidence is obtained

TABLE 54

Human Transmission Experiments with Urine from Cases of Infective Hepatitis

Experiment No	No of donors in pool	Time of collection of urine	Route of administration	Amount (ml)	No of recipients	No of recipients becoming jaundiced	Incubation period (days)
13	6	Day of onset of jaundice	Nasal and oral	9	19	0	-

TABLE 55

Human Transmission Experiments with Faeces from Cases of Infective Hepatitis

Experiment No	No of donors in pool	Time of collection of faeces	Route of administration	Amount (ml)	No of recipients	No of recipients becoming jaundiced	Incubation period (days)
14	2	Day of onset of jaundice 3rd day of jaundice	Nasal and oral	4	6	0	-
15	2	Day of onset of jaundice 3rd day of jaundice	Nasal and oral	4	6	0	-
16	4*	Day of onset of jaundice 3rd day of jaundice	Nasal	2	2	2	27 31
17	3	Pre icteric 5th–6th day of symptoms	Nasal and oral	4	7	1	27
18	2	One on 2nd day of symptoms and day before jaundice one on day of onset of jaundice	Nasal and oral	4	5	0	-
19(a)	Remains of filtrate from donors in experiment Nos 14 15 17 and 18 plus 2	One on 4th day of symptoms and day before jaundice one sub-icteric 3rd day of symptoms	Nasal and oral	3 10	20 (19(a) and (b))	0	-
(b)	1	On 1st day of jaundice inoculated 7 days after above pool into same donors	Oral	5			
20	2	730$\mu\mu$ filtrate of 19(a)	Nasal and oral	10	20	0	-
21	2	Filtrate of 19(a)	Parenteral	1·0	20	0	-

* Pool of remains of suspensions used in Experiments Nos 14 and 15

The only consistently positive transmission experiments have resulted from the oral administration of faecal suspensions (or their Seitz filtrates) or from the parenteral inoculation of serum, these experiments have been confirmed now with material from England, U S A, Italy, North and West Africa In the experiments in the U S A (Havens *et al*, 1944, Neefe *et al* 1945) and in West Africa (Findlay and Willcox, 1945) a higher proportion of the recipients developed hepatitis than in our experiments, and, as already suggested, this difference may have been due either to inactivation or attenuation of the virus by the ether treatment of the faeces, to a greater inherent degree of resistance of the individuals used in England or, as seems most likely, to the older age of the English recipients Liver dysfunction is exceedingly common among the natives of West Africa, and this may have influenced the susceptibility of the recipients used by Findlay Neefe and his colleagues have described an epidemic in a summer camp, in which there was strong evidence for faecal contamination of well water as the source of infection It seems probable that the widespread scattering of infected faeces and passive transfer by flies may have been the mode of transmission under battle conditions in the North African desert and in other epidemics in the Middle East during 1942 and 1943 But it is difficult to believe that this is the mode of transmission in the usual urban or small rural outbreak in England, or that it is responsible for the large number of apparently isolated sporadic cases

The earliest positive samples of faeces tested in our experiments were obtained 5 days after the onset of symptoms and the latest 8 to 10 days after the onset of symptoms and 24 to 96 hours after the appearance of jaundice The latest recorded positive specimen was one used by Havens *et al* (1944) which had been collected 8 days after the appearance of jaundice Neefe, Stokes and Reinhold (1945) were unable to infect volunteers with faeces collected 3 weeks after the disappearance of jaundice, and Havens obtained negative results with faeces and serum collected 25 to 31 days after the onset of illness Though more experiments will be necessary before definite conclusions are possible on this point, these negative results suggest that individuals do not excrete the virus for long periods after recovery from infective hepatitis

Like Neefe *et al* (1945) and Havens (1946a) in the U S A, we have failed to find evidence of the virus in the urine Findlay and Willcox (1945) in West Africa obtained positive results, but on reinvestigation it was found that the donors of these urines were also suffering from urinary schistosomiasis (Findlay, 1948), so that the urine is probably only a source of infection when both these diseases occur at the same time

Biochemical tests have indicated liver damage as early as 16 hours after the onset of symptoms and 10 to 14 days before the appearance of icterus As the pathological process must have been in progress for some time to produce this detectable damage, it is probable that circulating virus could be detected several days before the onset of symptoms as has been done in cases of homologous serum hepatitis The positive results obtained with the blood of our sub icteric case (see Experiment No 10, Table 53) indicate that here is another possible source of infection for syringe transmitted hepatitis or hepatitis from pools of sera used for therapeutic purposes Francis, Frisch and Quilligan (1946) have recently described just such an incident

HOMOLOGOUS SERUM HEPATITIS

This term is used here to describe cases or series of cases which originated in the first instance from inoculation of whole blood, serum or plasma, either intradermally, subcutaneously, intramuscularly or intravenously, for transfusion or other therapeutic purposes It refers only to hepatitis which has developed 40 to 200 days (approximately) after inoculation and not to cases of jaundice immediately following transfusions The results of cross-immunity experiments in Great Britain and in the United States suggest that the agent usually responsible differs from the virus of infective hepatitis, and epidemiological evidence points to the same conclusion

MacCallum and Bauer (1944) earlier described experiments with an icterogenic pool of serum collected from supposedly normal donors at blood transfusion centres, as well as experiments with serum from a case (F) of hepatitis presumed to have been induced by this serum The main findings were that the pooled serum produced hepatitis with jaundice in about 40 per cent of recipients when injected subcutaneously, but had no effect when administered intranasally There was no evidence that the agent in the pooled serum survived passage in chick embryos or tissue culture, nor did it produce any abnormalities in experimental animals The agent produced hepatitis in one of two men who had recovered from an attack of infective hepatitis 6 months previously, but had no effect on eight men who were convalescent from homologous serum hepatitis induced in the United States and on two in whom it had been induced in England On the other hand the serum from the single induced case, F, produced hepatitis and jaundice when given to volunteers intranasally, and there was suggestive evidence that it had survived, if not multiplied, in tissue culture In the light of the results of experiments done since that time in the United States and in this country, the nature of case F is still in doubt

Experiments have been made to obtain further information regarding another icterogenic pool, Batch 034, composed of serum collected from approximately one thousand supposedly normal donors at blood banks Samples of this pool had already produced jaundice in 57 per cent of a group of seventy-one recipients injected with it in a special investigation before its icterogenic nature was recognized As well as attempting to determine the possible relation between the causative agents of homologous serum hepatitis and infective hepatitis, it was considered of great importance to find a routine method of inactivating all such pools in order to render them non-icterogenic

Experimental Methods

Preparation and administration of serum The pool Batch 034, was a large one, the serum had been dried in 400 ml amounts and stored at +2°C Two bottles of widely separated numbers in the pool were chosen for subcutaneous and oral administration The sera were reconstituted in sterile distilled water, redried in 10 ml amounts for convenience and stored at +2°C till used For oral and subcutaneous administration each ampoule was reconstituted in sterile distilled water Five ml was sprayed either orally or intranasally and swallowed, and 1 0 ml was injected subcutaneously

Ether extraction of serum The serum contents of several bottles were reconstituted, pooled and then exposed by Dr R A Kekwick to triple extraction with ether at −20°C (McFarlane, 1942) This pool was again

dried in large amounts, later reconstituted once more, and redried in 10 ml amounts The serum was reconstituted for subcutaneous injection into volunteers in 1 0 ml amounts

Ultraviolet irradiation of serum Two experiments were made using two bottles of serum, the bottles being irradiated by different methods Both these bottles were also tested untreated

In the first experiment, which was relatively crude, the reconstituted serum was exposed in a quartz flask, with its round bottom resting on the ultraviolet lamp, the latter had a mercury argon low-pressure quartz discharge tube giving approximately 80 per cent of the total light radiation at the 2537 Å mercury resonance line One ml of the serum was placed in the flask at a time and was irradiated for 30 minutes, the flask being agitated by hand at frequent intervals The layer of serum was only 4 mm at the deepest point Unfortunately, it did not prove possible to carry out an electrophoretic examination of this serum after treatment

In the second attempt at inactivation by ultraviolet light, a different bottle of serum and a different apparatus were used The serum was reconstituted in the usual way and then exposed to a "Thermo Syndicate New Home Unit" lamp T/M5/369, which was 15 cm long and emitted 95 per cent of its energy at 2537 Å The serum was flowing through a coil with 30½ turns, the coil was 9 5 cm in diameter, 6 5 cm high, and 400 cm long, the inside diameter of the tube being 1 5 mm The lamp was surrounded by the coils The temperature of the serum was 30°C during exposure The serum was run through the coil twice, being exposed for 15 minutes each time A sample of this serum was examined electrophoretically by Dr Kekwick who states the "examination in the electrophoresis apparatus in a buffer pH 8 revealed that gross changes had occurred in the proteins as a result of irradiation The albumin was reduced in amount and instead of the usual α, β, γ globulin components, there was a single component migrating close to the albumin and accounting for about 60 per cent of the total protein It would seem probable that this component is a complex of albumin with all the globulins A very small residue of probably unchanged globulin was detectable"

The serum was kept frozen at −20°C till used, when 1 0 ml amounts were injected subcutaneously There was an immediate red flare at the site of inoculation but none of the recipients complained of any untoward symptoms

Tissue culture experiment One ml of reconstituted serum from a bottle from the pool was added to each of four flasks containing minced chick embryo tissue and 4 0 ml guinea pig serum-Tyrode's solution and incubated at 37°C Transfers were made every 3 or 4 days to fresh flasks, using 1 0 ml supernatant as was done in the yellow fever tissue cultures originally suspected of containing an infective agent by Findlay, MacCallum and Murgatroyd (1939) The remaining supernatant was dried *in vacuo* in 1 0 ml amounts in ampoules and stored at 0°C The dried material of the 5th and 10th subcultures was reconstituted in 1 0 ml sterile distilled water and injected subcutaneously into volunteers

It was not possible to carry out passage in chick embryos due to scarcity of fertile eggs

Animal inoculation experiments Only two series of animal experiments were performed with the serum from Batch 034 One consisted of the inoculation

of seventeen white rats of the Wistar strain; these animals were on a normal diet and receiving long courses of neoarsphenamine. The serum was given repeatedly intranasally, orally, intraperitoneally and intrahepatically, and the drug was given intraperitoneally. Rats were killed at intervals, from 30 to 157 days after the first inoculation but no passages were made. No histological abnormalities were detected.

The other series consisted of the blind passage of organs of rats inoculated with serum from Batch 034. The rats were on the same protein-deficient diet as had been used for the experiments with infective hepatitis material (see p. 113). Eight passages at 21- to 28 day intervals failed to produce any apparent disease in the rats. Six passages at 60 day intervals also failed to produce any histological abnormalities or disease.

Results

As in the experiments with infective hepatitis material the groups at risk were small, and it was considered that a statistical approach was not justifiable. The results of the experiments (Table 56) can therefore only be discussed in a general way and some suggestions offered as to probable deductions which can be made from them.

Though it was not possible to perform experiments which would give information about the transmission of homologous serum hepatitis by nasopharyngeal washings or faeces, some information was obtained with regard to the properties of an agent causing this disease.

It has already been established (MacCallum and Bauer, 1944) that the icterogenic agent of serum hepatitis can withstand heating at 56°C for an hour in a water-bath, and can survive for at least 14 months in the dried state and through several cycles of freezing, thawing, reconstitution and drying.

TABLE 56

Human Transmission Experiments with Icterogenic Serum (Batch 034)

Material		*Bottle*	*Amount (ml)*	*Route of administration*	*No of recipients*	*No of recipients becoming jaundiced*	*Incubation period (days)*
Untreated dried serum		161	1	Parenteral	4	3	60–114
		224	1	Parenteral	14	6	69–112
		224	5	Oral	10	0	–
		224	5	Nasal	10	0	–
Serum after triple ether extraction		150–159	1	Parenteral	19	10	83–131
Serum after irradiation with ultra-violet light	(1)	224	1	Parenteral	10	2	90, 99
	(2)	161	1	Parenteral	10	1	27
Serum after tissue culture	5th sub-culture	140	1	Parenteral	10	0	0
	10th sub-culture	140	1	Parenteral	10	0	0

Serum from Batch 034, which had produced jaundice in 50 to 60 per cent of different groups who had received small amounts subcutaneously, failed to produce any clinical signs of hepatitis in twenty individuals who each received 5 ml of the material by the nasopharyngeal or oral routes These findings agree with those of workers in the United States It must be admitted that we also failed to produce hepatitis by oral administration of one pool of blood from two cases of infective hepatitis (see p 123) but this had been done repeatedly by others so that the weight of evidence suggests that serum from cases of infective hepatitis will usually produce hepatitis when given orally and so differs from the agent or agents causing serum hepatitis

Triple ether extraction of the serum in the cold produced a very clean, transparent product which was admirable for injection, but unfortunately this treatment apparently had no inactivating effect on the icterogenic agent, ten out of the nineteen recipients becoming jaundiced

The results of experiments by Oliphant, Gilliam and Larson (1943), Oliphant (1944a) and Oliphant and Hollaender (1946) in the United States suggested that ultraviolet light of certain wave-lengths might inactivate the icterogenic agent in the serum if the latter was exposed as a thin film of moving liquid In several of their experiments the icterogenic rate in the volunteers who received the non-irradiated serum was very low and did not permit a definite conclusion as to the efficacy of irradiation Later a more satisfactory experiment by Blanchard, Stokes, Hampil, Wade and Spizizen, (1948) confirmed that ultraviolet light was capable of inactivating an icterogenic agent in serum

In our first rather crude experiment with ultraviolet light two out of ten recipients of the irradiated serum developed jaundice, showing that inactivation had not taken place

In the second experiment, with the different type of apparatus and exposure, one out of the ten individuals who received this treated serum developed jaundice However this patient, who was aged 43 and suffered from rheumatoid arthritis, became jaundiced 27 days after inoculation, also she was resident in a large city and the possibility of contact with a case of infective hepatitis cannot of course be excluded The incubation period of 27 days is about 20 days less than that of any other individual who received this batch of icterogenic serum, however administered or previously treated, which throws some doubt on the injected serum being the cause of her hepatitis There have been, however, occasional instances of single individuals developing hepatitis after a very short incubation period in other large groups of persons inoculated from pools of icterogenic serum, so that the case cannot be ignored

As already stated, electrophoretic examination of the serum irradiated by the second method revealed that gross changes in the proteins had occurred

The serum in this case was apparently over irradiated and later results (Blanchard *et al*, 1948) showed that when an icterogenic serum was exposed as a thin moving film, irradiation by the appropriate dose of ultraviolet light inactivated the icterogenic agent without altering the normal proteins present in the serum

In 1939 Findlay and his colleagues suggested that the icterogenic agent present in yellow fever vaccine at that time might have been propagated in their tissue cultures Using the same experimental conditions we have failed to obtain any evidence of the growth of the agents in two batches of

serum, 207Ky (MacCallum and Bauer, 1944), and 034 which was used in the present investigation

ARSENOTHERAPY HEPATITIS

Hepatitis was very prevalent in certain clinics for the treatment of venereal diseases at the time this investigation was begun In contradiction to the theory that this disease was due entirely to toxicity of the arsenical drug, it had been suggested by Bigger (1943) and MacCallum (1943) that the disease was usually due to injection and passage of an icterogenic agent present in the blood of some of the men under treatment, the drug *per se* playing no part It was suggested that the passage by injection was made possible by the shortage of syringes and staff in the clinics which resulted in the same syringe being used for a number of individuals without adequate sterilization between each injection The icterogenic agent was presumed to be active in minute amounts and very resistant to short exposure to ordinary disinfectants and heat Strong support for this hypothesis was provided by the field experiments of Salaman, King, Williams and Nicol, (1944) who used individual dry-heat sterilized syringes and needles for one group of patients and the usual less efficient methods prevailing at the time for a second group at the same clinic They found the incidence of hepatitis in the second group was many times that in the first Clearly it was possible that similar conditions for transmission might exist wherever syringes were used on more than one patient without proper sterilization

It was decided to carry out the same type of transmission experiments as had been made in studying infective hepatitis in order to try to throw some light on the problem The methods used are described below and the results are shown in Table 57 These experiments have been described briefly in a preliminary note (MacCallum 1945)

Experimental Methods

Blood Serum was collected on the first day of icterus from two patients, Ar I and Ar II, who became jaundiced during arsenical treatment in two clinics where there had been a large number of such cases The first patient, Ar I, became jaundiced during the 14th week of a course of weekly injections of neoarsphenamine, and the second, Ar II, 110 days after the first injection and 82 days after the last injection of a course of intensive treatment with a similar type of drug

Ten volunteers were injected subcutaneously with 0 25 to 1 0 ml serum from Ar I (Ar SI) and nine volunteers with 0 25 ml serum from Ar II (Ar SII)

Faeces Faeces were collected from Ar II at the same time as the serum and were stored at −20°C A suspension was prepared and administered in the same manner as the material from cases of infective hepatitis (see p 119) All fifteen recipients in this group and five additional individuals also received a dose of untreated faeces from four cases of hepatitis induced by inoculation of Ar SI

Nasopharyngeal washings Nasopharyngeal washings were not available from donors Ar I and Ar II, but as soon as the first case of hepatitis induced by Ar SI occurred we commenced transmission experiments with nasopharyngeal washings from all those who had received this serum (Table 57)

The material was administered within an hour of collecting in every case, and the techniques of collection and administration were the same as in the infective hepatitis experiments (p 118) There were seven recipients of Ar SI who developed jaundice and contributed nasopharyngeal washings garglings and tonsillar swabs from 31 days before to 4 days after the appearance of their jaundice Each pool was collected from one to five donors (Table 58) There were seventeen recipients of whom five received material from only one pool, three received material from two, eight received from three, and one from four pools (Table 59)

All the volunteers were between the ages of 30 and 60, except two aged 18 and 24 who received Ar SII, one aged 20 who received faeces, and two aged 23 and 29, who received nasopharyngeal washings

Results

Serum Each serum contained an extremely active icterogenic agent which when injected subcutaneously, produced hepatitis in seven out of ten and five out of nine recipients respectively after intervals scattered evenly between extremes of 41 to 80 days (Table 57) These results suggest that the

TABLE 57

Human Transmission Experiments in Arsenotherapy Hepatitis

Material	*Source*	*No of recipients*	*No of recipients becoming Jaundiced*	*Incubation period (days)*
Serum Ar SI	Patient Ar I 1st day of jaundice	10	7	41–72
Ar SII	Patient Ar II 1st day of jaundice	9	5	45–80
Faeces Ar FI	Patient Ar II and 4 patients induced by Ar SI	15	0	–
Ar FII	4 patients induced by Ar SI	5	0	–
Nasopharyngeal washings	7 patients induced by Ar SI	17	2	100 and 104

hepatitis of Ar I and Ar II had not been caused by the arsenicals injected The incubation periods give little clue as to whether the donors' hepatitis was caused by infection with or injection of the agent of infective hepatitis or injection of an agent of serum hepatitis The fact that none of the recipients had an incubation period of less than 41 days, and that Ar II had had infective hepatitis 12 months before the illness investigated by us and before he contracted syphilis suggests that the diagnosis was homologous serum hepatitis at least in his case The high attack rate for recipients aged 30 to 60 inoculated subcutaneously is also in favour of serum hepatitis The husband of one induced case developed jaundice 63 days later We hoped that the application of the complement fixation test described by Miles (1946 and see p 103 of this Report) for sera from cases of arsenotherapy hepatitis would help to identify the aetiological agent in these cases But unfortunately a satisfactory antigen was no longer
[illegible] sera from Ar I and Ar II and the recipients
any conclusion to be
the two cases studied

TABLE 58

Details of Donors contributing to Pools of Nasopharyngeal Washings

Nasal washings		*Donor panel*	
Pool No	*Donors*	*Donor No*	*Date of onset of donor s jaundice*
I	z	z	18 12 44
II	z	y	18 1 45
III	z, y, x, w, v	x	30 12 44
IV	z, y, t	w	2 1 45
V	s	v	12 1 45
VI	s	t	17 12 44
VII	x, y	s	22 12 44

Faeces Transmission experiments with faeces gave entirely negative results in the group of twenty recipients, five of whom were under 39 years of age and fifteen between 44 and 57 years

TABLE 59

Results of Transmission Experiments with Nasopharyngeal Washings from Cases of Hepatitis Induced by Serum from a Case (Ar I) of Arsenotherapy Hepatitis

Pool No	I	II	III	IV	V	VI	VII
Date of collection	*18 12 44*	*19 12 44*	*20 12 44*	*22 12 44*	*22 12 44*	*23 12 44*	*28 12 44*
Recipients	a	–	a		a	–	
	–	b	b	–	b	–	b
	–	–	c	c		c	–
	–	–	d	d	–	d	–
	–	–	e	e			e
	–	–	f	f		f	–
	–	–	g	g		g	
	–		h	h		h	–
	–	–	i	i		i	
	–	–	j	–	–	–	–
	–	–		k		k	–
	–	–	–	m	–	m	–
	–	–			n	–	n
	–	–	–	–	o		
	–	–	–		–	–	p
	–	–	–				q
	–	–	–	–	–	–	r

Note —Recipients **f** and **k** became jaundiced

Nasopharyngeal washings In a preliminary communication (MacCallum, 1946), made 3 months after the administration of the nasopharyngeal washings this group was also described as negative Subsequently, two of the seventeen recipients (**f** and **k** in Table 59) became jaundiced 100 and 104 days respectively after their first inoculation **f** received nasopharyngeal washings from three pools containing material collected 31 days before to 4 days after the appearance of icterus in five donors, **k** two inoculations from pools collected 27 days before to 4 days after the onset of jaundice from four donors They both received material from Pools IV and VI The former was obtained from three donors on the 27th and 7th days before and

the 4th day after, the appearance of jaundice respectively. Pool VI was derived from a single donor the day after the appearance of jaundice Material from these pools was administered to seven and six other recipients respectively It is regrettable that serum from these donors was not collected at the same time as the nasopharyngeal washings in order to test for circulating virus, but an experiment by Paul Havens, Sabin and Philip (1945) has shown that the causative agent of homologous serum hepatitis was circulating in the blood stream 30 days after the inoculation and 64 days before jaundice With this in mind, and recalling our negative results with nasopharyngeal and oropharyngeal washings from cases of infective hepatitis (see p 120), one is tempted to incriminate the pool containing the material collected from a patient 27 days before the appearance of jaundice. There is of course the possibility that both these recipients contracted infections of infective hepatitis, but there were no known cases in friends or relatives

Discussion

Though the amount of material tested and the number of recipients were small these results indicate that the incubation period of so called arsenotherapy hepatitis following parenteral injection of blood corresponds to that of hepatitis following injection of serum from cases of infective hepatitis and homologous serum hepatitis, this finding gives no clue as to the nature of the original donor's disease The fact that serum from a donor, Ar II, who had had infective hepatitis about a year before the onset of his arsenotherapy hepatitis produced a high attack rate in adults, and the negative results with faeces, suggest that the illness of Ar II, at least, was homologous serum hepatitis The high attack rate following the injection of the sera Ar SI and Ar SII, together with the results of the field experiments of Salaman and his colleagues (1944) provide the proof of the hypothesis on which both types of experiment were based, i e that arsenotherapy hepatitis in a V D clinic is usually the result of the injection of an icterogenic agent present in the blood of one or more individuals attending for treatment The agent is extremely resistant to heat and the weaker antiseptics and cases of hepatitis occur when the same syringe is used for treating a number of individuals without adequate sterilization between individual bleedings and injections

GLANDULAR FEVER

It has been noted frequently in recent years that certain cases of glandular fever became jaundiced in the course of their illness It was originally suggested that this was the result of obstruction of the common bile duct by enlarged glands but more recent biopsy studies of the liver have indicated that the lesion is intrahepatic and resembles that seen in infective hepatitis or homologous serum hepatitis There has been a suggestion that glandular fever is related in some way to infective hepatitis

In the course of our investigation several cases of glandular fever with jaundice were seen The serum from one such patient collected on the day of onset of jaundice was separated from the cells and 0 75 ml was injected subcutaneously into each of six volunteers None of the volunteers developed either hepatitis or glandular fever

General Discussion

In the preceding pages we have used the terms in common use for describing the various types of hepatitis under discussion i e infective

hepatitis, homologous serum hepatitis and arsenotherapy hepatitis Arsenotherapy hepatitis is considered as representative of a group for which the term syringe-transmitted hepatitis has been suggested recently We believe there are two different agents responsible for the first two types As either agent may be present in the serum of an individual and so cause serum-, transfusion-, or syringe-transmitted hepatitis it is proposed for the purpose of clarity in discussion to call the agent of infective hepatitis, hepatitis virus A, and that of homologous serum hepatitis, hepatitis virus B

It has already been stated that the small number of positive takes in our human volunteer experiments render these unsuitable for statistical analysis This limitation must be kept in mind when reading the following discussion of the results of the experiments reported here and of similar experiments carried out by Findlay and his colleagues (1943-45), Havens and his colleagues (1944 46) and Neefe and his colleagues (1945-46)

It is now generally accepted that infective hepatitis (epidemic hepatitis) is caused by a virus (hepatitis virus A) Though claims of successful transmission of the virus to various animals have been made, these have not been satisfactorily substantiated Our experiments on Wistar-strain white rats on a protein deficient diet suggest a possible approach to the problem We have described what appears to be a transmissible disease in these rats, but the source of the causative agent has not been determined The disease failed to appear consistently in later passages so that satisfactory neutralization tests with human hepatitis convalescent serum were impracticable Transmission of the disease failed to occur after the 12th passage Three further attempts to reproduce the disease by blind passage of organs of rats inoculated with fresh, presumably infective, faeces and blood from cases of infective hepatitis have also been unsuccessful

As a result of the failure of animal transmission experiments recourse has been made to human volunteers in the United States and in England We have obtained evidence of the presence of virus in faeces from cases of infective hepatitis from several days before the appearance of jaundice to 4 days after (Table 55, p 125) Similar results have been obtained by Findlay and Willcox (1945), Havens *et al* (1944), and Neefe *et al* (1945), with the latest positive stool collected on the eighth day after the appearance of jaundice Havens and Neefe failed to obtain evidence of the persistence of virus in the serum or stools 25 to 31 days after the onset of illness in individuals from whom positive specimens were obtained during the early stages of the disease Findlay and Willcox found the virus also in the urines of patients who were suffering from urinary schistosomiasis as well All our attempts to demonstrate the presence of virus in the nasopharynx and oropharynx after the onset of symptoms have failed (Table 60, p 136) It is possible that the virus is present in the nasopharynx and oropharynx some time during the incubation period of the disease (see p 36), but this hypothesis is as yet unproven There have been a certain number of epidemics which, on epidemiological grounds (i e the presence of an excessive number of flies, insanitary conditions and drinking water contaminated with faecal organisms) appear to have been spread by ingestion of food or water contaminated by faeces containing virus. In the majority of epidemics, however, especially those studied in England the spread infection appears to have been by direct contact. All [illegible]
clinical experiments to date indicates that the faeces [illegible] son

the 4th day after, the appearance of jaundice respectively. Pool VI was derived from a single donor the day after the appearance of jaundice Material from these pools was administered to seven and six other recipients respectively It is regrettable that serum from these donors was not collected at the same time as the nasopharyngeal washings in order to test for circulating virus, but an experiment by Paul, Havens, Sabin and Philip (1945) has shown that the causative agent of homologous serum hepatitis was circulating in the blood stream 30 days after the inoculation and 64 days before jaundice With this in mind, and recalling our negative results with nasopharyngeal and oropharyngeal washings from cases of infective hepatitis (see p 120), one is tempted to incriminate the pool containing the material collected from a patient 27 days before the appearance of jaundice There is of course, the possibility that both these recipients contracted infections of infective hepatitis, but there were no known cases in friends or relatives

Discussion

Though the amount of material tested and the number of recipients were small these results indicate that the incubation period of so called arsenotherapy hepatitis following parenteral injection of blood corresponds to that of hepatitis following injection of serum from cases of infective hepatitis and homologous serum hepatitis, this finding gives no clue as to the nature of the original donor's disease The fact that serum from a donor, Ar II, who had had infective hepatitis about a year before the onset of his arsenotherapy hepatitis, produced a high attack rate in adults, and the negative results with faeces, suggest that the illness of Ar II, at least, was homologous serum hepatitis The high attack rate following the injection of the sera, Ar SI and Ar SII, together with the results of the field experiments of Salaman and his colleagues (1944), provide the proof of the hypothesis on which both types of experiment were based, i e that arsenotherapy hepatitis in a V D clinic is usually the result of the injection of an icterogenic agent present in the blood of one or more individuals attending for treatment The agent is extremely resistant to heat and the weaker antiseptics, and cases of hepatitis occur when the same syringe is used for treating a number of individuals without adequate sterilization between individual bleedings and injections

GLANDULAR FEVER

It has been noted frequently in recent years that certain cases of glandular fever became jaundiced in the course of their illness It was originally suggested that this was the result of obstruction of the common bile duct by enlarged glands but more recent biopsy studies of the liver have indicated that the lesion is intrahepatic and resembles that seen in infective hepatitis or homologous serum hepatitis There has been a suggestion that glandular fever is related in some way to infective hepatitis

In the course of our investigation several cases of glandular fever with jaundice were seen The serum from one such patient collected on the day of onset of jaundice was separated from the cells and 0 75 ml was injected subcutaneously into each of six volunteers None of the volunteers developed either hepatitis or glandular fever

General Discussion

In the preceding pages we have used the terms in common use for describing the various types of hepatitis under discussion, i e infective

hepatitis, homologous serum hepatitis and arsenotherapy hepatitis Arsenotherapy hepatitis is considered as representative of a group for which the term syringe transmitted hepatitis has been suggested recently We believe there are two different agents responsible for the first two types As either agent may be present in the serum of an individual and so cause serum, transfusion, or syringe transmitted hepatitis it is proposed for the purpose of clarity in discussion to call the agent of infective hepatitis hepatitis virus A, and that of homologous serum hepatitis, hepatitis virus B

It has already been stated that the small number of positive takes in our human volunteer experiments render these unsuitable for statistical analysis This limitation must be kept in mind when reading the following discussion of the results of the experiments reported here and of similar experiments carried out by Findlay and his colleagues (1943 45) Havens and his colleagues (1944 46) and Neefe and his colleagues (1945 46)

It is now generally accepted that infective hepatitis (epidemic hepatitis) is caused by a virus (hepatitis virus A) Though claims of successful transmission of the virus to various animals have been made, these have not been satisfactorily substantiated Our experiments on Wistar strain white rats on a protein deficient diet suggest a possible approach to the problem We have described what appears to be a transmissible disease in these rats but the source of the causative agent has not been determined The disease failed to appear consistently in later passages so that satisfactory neutralization tests with human hepatitis convalescent serum were impracticable Transmission of the disease failed to occur after the 12th passage Three further attempts to reproduce the disease by blind passage of organs of rats inoculated with fresh, presumably infective faeces and blood from cases of infective hepatitis have also been unsuccessful

As a result of the failure of animal transmission experiments recourse has been made to human volunteers in the United States and in England We have obtained evidence of the presence of virus in faeces from cases of infective hepatitis from several days before the appearance of jaundice to 4 days after (Table 55, p 125) Similar results have been obtained by Findlay and Willcox (1945) Havens *et al* (1944), and Neefe *et al* (1945) with the latest positive stool collected on the eighth day after the appearance of jaundice Havens and Neefe failed to obtain evidence of the persistence of virus in the serum or stools 25 to 31 days after the onset of illness in individuals from whom positive specimens were obtained during the early stages of the disease Findlay and Willcox found the virus also in the urines of patients who were suffering from urinary schistosomiasis as well All our attempts to demonstrate the presence of virus in the nasopharynx and oropharynx after the onset of symptoms have failed (Table 60 p 136) *It is possible that the virus is present in the nasopharynx and*

excessive number of flies, insanitary conditions and drinking water contaminated with faecal organisms) appear to have been spread by ingestion of food or water contaminated by faeces containing virus In the majority of epidemics however, especially those studied in England the spread of infection appears to have been by direct contact. All the evidence from our clinical experiments to date indicates that the faeces are the source of the

virus for such infections There have been several reports of epidemics of infective hepatitis in which it has been suggested that the source of infection has been an individual in a relapse from infective hepatitis Unfortunately *there is no experimental evidence for* or against this hypothesis

The virus has been detected circulating in the blood stream of an induced case of infective hepatitis as early as 3 days before the first symptom and 13 days before the onset of jaundice (Francis, Frisch and Quilligan, 1946) The virus has been found in the blood as late as 8 days after the onset of symptoms, but not 31 days after the onset of symptoms (Havens, 1946a)

TABLE 60

Summarized Results of Human Transmission Experiments

Material	*Route of administration*	[illegible]	[illegible]	[illegible]
Serum	Oral	+ Havens *et al* (1944) Neefe *et al* (1946)	– Havens (1946b) MacCallum (1946)	Not tested
	Subcutaneous	+ Cameron (1943) MacCallum and Bradley (1944) Havens *et al* (1944)	+ Oliphant *et al* (1943) MacCallum and Bauer (1944)	+ MacCallum (1945)
Nasopharyngeal washings	Oral	– MacCallum and Bradley (1944) *Neefe et al* (1946) Havens (1946b)	+ Findlay and Martin (1943)	+* MacCallum (present report)
Faeces	Oral	+ MacCallum and Bradley (1944) Havens *et al* (1944)	– Neefe *et al* (1945)	– MacCallum (1945)
Urine	Oral	+ † Findlay and Willcox (1945)	Not tested	Not tested

* Only one strain tested
† These patients were also suffering from urinary schistosomiasis (p 126)

It is interesting to note that there have been several instances when sera collected from typical cases on the day of appearance of *jaundice have failed* to produce disease in groups of five or six recipients when inoculated subcutaneously (Table 53, p 122, and Neefe, Stokes and Gellis, 1945)

The question of *the duration of the presence* of the hepatitis virus A in the blood is directly related to the problem of the disease at present known as homologous serum hepatitis It is now recognized that certain pools of serum and plasma from apparently healthy individuals may contain an icterogenic [illegible] produces a disease clinically indis-
whether the causative
Our experiments during
have demonstrated the

presence of an infective agent, which was probably virus A, in the blood of an individual with hepatitis, who at the time was asymptomatic and his serum bilirubin only 0.25 mg. per 100 ml. (see p. 123). Francis *et al.* (1946) have described the case of a soldier stationed in Italy when infective hepatitis was epidemic, who was in good health when he donated 500 ml. of whole blood for a transfusion, 2 days before the onset of his symptoms and jaundice. He was considered to be a case of infection with virus A. The recipient developed symptoms of hepatitis 11 days later and jaundice 21 days after transfusion. Blood collected from the recipient 3 days before the onset of symptoms and 13 days before the appearance of jaundice contained an icterogenic agent. These results indicate that it is possible for an individual who is symptomless but carrying virus A (of infective hepatitis) in his blood to render a pool of blood icterogenic.

TABLE 61

Results of Cross immunity Experiments by Various Authors

Author	Nature of first attack in subjects reinoculated	Result of reinoculation with material from cases of homologous serum hepatitis		Result of reinoculation with material from cases of infective hepatitis	
		No. inoculated	No. developing jaundice	No. inoculated	No. developing jaundice
Beeson Chesney and McFarlan (1944)	Infective hepatitis in childhood	11	8	—	—
Havens (1945)	Experimental homologous serum hepatitis	—	—	3	3
MacCallum and Bauer (1944)	Homologous serum hepatitis	10	0	—	—
	Natural attack of infective hepatitis	2	1	—	—
MacCallum, Stewart and Bradley (present report)	Homologous serum hepatitis	—	—	2	2
Neefe Stokes and Gellis (1945)	Experimental homologous serum hepatitis	6	0	6	2 (+3)*
	Experimental infective hepatitis	—	—	8	0
Oliphant (1944b)	Experimental homologous serum hepatitis	10	0	—	—
	Experimental homologous serum hepatitis †	—	—	10	0

* These three cases showed hepatitis but no jaundice.
† See text for possible explanation of the anomalous result in this experiment.

The results of administering to volunteers serum, nasopharyngeal washings and faeces from cases of infective hepatitis (virus A), homologous serum hepatitis (virus B) and syringe-transmitted hepatitis are shown in Table 60, and the results of immunity and cross-immunity experiments in Table 61. These results indicate that in the majority of instances the icterogenic agent present in the serum or plasma causing homologous serum hepatitis is not virus A (of infective hepatitis). Virus A has been found in the faeces and urine. Virus B has not been found in the faeces in naturally-occurring cases of homologous serum hepatitis or in experimentally-induced cases even when

the donor serum was injected subcutaneously It has not been tested for in the urine Virus A has not been found in the nasopharyngeal washings, but isolation of virus B has been claimed by *Findlay and Martin (1943)*, we also obtained apparent transmission of an agent in nasopharyngeal washings which probably was virus B, but after a much longer incubation period (see p 134) In a Division of the American Army in the Mediterranean theatre a group of 438 individuals convalescent from one type, virus B hepatitis, seemed to be 2½ times more susceptible to virus A than did 2,519 normal controls (Gauld, 1946) Pooled serum from cases of virus A hepatitis has been infective when given by the oral or parenteral route, but pooled serum containing virus B has only been infective by the parenteral route Results similar to our own with pooled serum containing virus B have recently been reported by Havens (1946b) Experimental reinoculations with homologous and heterologous agents (Neefe *et al*, 1945, Havens, 1945) have indicated almost solid immunity to the homologous agent, but none to the heterologous agent, except in the experiment described by Oliphant (1944b), (see Table 61) In his case the patient was presumed to be suffering from infective hepatitis in Italy but may have had virus B in his blood

If we accept that there are two agents, A and B, which cause a similar clinical disease, it is difficult to determine without a confirmatory laboratory test which agent is involved when an individual who has not received an injection of blood develops hepatitis To date, all such cases have been presumed to be virus A infections All studies of virus B infections have originated from pools of icterogenic sera, but so far no donor known to have had a recent attack of hepatitis has been traced as having contributed to these pools No case of hepatitis of either type examined experimentally has been shown to be carrying an icterogenic agent in his blood as long as a month after his hepatitis So far, no complete study has been made of a subject with hepatitis, who has not received any type of injection which might have been responsible, whose blood has been infective after a long incubation period (60 to 150 days) when injected parenterally, and whose serum and faeces have failed to produce disease when administered orally—the present criteria of virus B infection How then is infection with virus B transmitted other than by injection, for it is unlikely that the virus could be maintained in the community by *this method alone? Findlay and Martin (1943) claim to* have caused hepatitis in three volunteers by the oral administration of nasopharyngeal washings from three cases of presumed virus B infections, the *incubation periods* in these cases were 28, 30 and 50 days respectively This is a very high attack rate and does not agree with observation in the *field, but it may* have resulted from the particular circumstances of their experiments Two cases of hepatitis occurred after incubation periods of about 100 days among our group of seventeen volunteers who received nasopharyngeal washings from cases of hepatitis which were probably caused by virus B (patient Ar 1 see p 132) The high attack rate among adults inoculated with pools of sera presumed to contain virus B suggests that only a small proportion of the population has been exposed to this agent as compared to virus A in England The presumption is therefore that if homologous serum hepatitis is *truly infectious, it is* either a new disease or natural transmission is very difficult It is not known whether the apparent increase in incidence of the disease is due to its better recognition, or to an actual increase *in the number* of cases as a result of the widespread use of

blood products, which in turn may have caused naturally-occurring cases from contact infection If there has been an increase in such natural infections among uninoculated individuals can the increase be related in any way to the recent epidemics of virus A infection? Could such an increase be related to the reaction of the host to virus A, with production of a new variant of the virus or a new antibody in the serum? Patients who have had virus B hepatitis and apparently recovered seem to be more susceptible to infection with virus A than do normal individuals But McKinlay and Truelove (1947) found that a previous attack of infective hepatitis did not affect the liability to arsenotherapy hepatitis

Thus in spite of the information which has accrued from these experiments in human volunteers the derivation of virus B and its possible relation to virus A remains unsolved

Both agents, if we consider there are two, are very resistant to the ordinary methods of inactivation And at present the only satisfactory routine method of rendering sera non icterogenic is by ultraviolet irradiation Recent observations indicate that even this method may not always be effective as a routine with the equipment in use in certain centres (James, Korns and Wright, 1950)

We wish to acknowledge with thanks the help of Dr J Mitchell of Cambridge and Dr A S McFarlane of the National Institute for Medical Research in the ultraviolet irradiation experiments, and that of Dr R A Kekwick of the Lister Institute in providing the ether-extracted sera and carrying out electrophoretic examination of one of the irradiated sera

References

VII. SUMMARY

Epidemiological Studies

(1) Notifications of infective hepatitis, which were made compulsory in nine counties in the east of England with a total population of 2 6 millions (Region 4), showed a falling incidence from 1943 to 1947 interrupted by a slight rise in 1946

(2) The seasonal trend in 1943, 1944, 1945 and 1947 resembled that found in other countries with a fall from the beginning of the year to a minimum in summer and a rise beginning in autumn and continued in winter In 1946 there was a peak in early summer due to an epidemic in the southern part of the Region

(3) The age distribution of cases indicated a peak incidence in children aged 5 to 14 years The incidence in young adults was higher than that of some other common infectious diseases Homologous serum hepatitis has a different age incidence with a peak in adult life

(4) The incidence in urban districts was lower than that in rural districts in 1944, equal to it in 1945 and higher in 1946 and 1947

(5) Epidemics in towns and villages lasted for several months with a slow rise and fall In several large towns an epidemic was followed by an endemic prevalence, but in small towns and villages no cases were notified for months or years afterwards

(6) The geographical distribution of the disease in successive years suggested that the infection had spread centrifugally over the Region from London Centrifugal spread from a town to surrounding villages was observed in three areas

(7) The incubation period was estimated by several methods and the results confirmed earlier work by showing that it was about 20 to 40 days Infectivity is greatest at the time of onset of symptoms but some observations suggested that there may be a period of infectivity early in the incubation period

(8) Details of an outbreak in a mental institution are given

(9) Contact was the probable mode of spread Carriers are probably concerned in the spread, and epidemiological findings suggest that both pharyngeal secretions and faeces may transmit infection

Clinical Observations

(1) Clinical studies are reported on 2 000 sporadic and epidemic cases of infective hepatitis in Service personnel

(2) The common signs were jaundice, enlargement and tenderness of the liver splenomegaly, fever and bradycardia

(3) The majority of patients presented a combination of general and digestive symptoms Symptoms of upper respiratory infection appeared to be coincidental rather than an integral part of the disease

(4) It was not possible either in the sporadic or epidemic cases in this series to separate different clinical types of jaundice by the presence or absence of constitutional symptoms and on clinical grounds there appeared to be no evidence that "catarrhal jaundice" exists as a separate entity from infective hepatitis

(5) The course of the disease is divided into pre icteric icteric and post icteric stages During the pre icteric stage symptoms were most severe and

liver damage, in cases which followed the usual course to recovery i maximal Remissions occasionally occurred with dissociation of general digestive symptoms During the icteric stage both symptoms and liver f tion improved rapidly
frequently outlasted all
even more persistent
post icteric stage, some residual symptoms often persisted but these rarely severe enough to interfere with normal work Residual signs incl hepatic enlargement and tenderness, and splenomegaly

(6) Relapses might occur while the patient was still confined to b the absence of any obvious precipitating factor Intercurrent infe appeared to be without effect on the course of the disease

(7) A comparison of sporadic cases in home based units with epi cases in troops recently returned from the Mediterranean area reveal difference in symptomatology or in severity as judged by clinical or chemical criteria

(8) Cases of non icteric infective hepatitis encountered during the p investigation were very few, and depended on biochemical evidence fo diagnosis

(9) Although both symptoms and liver damage were usually maxi the early stages of the disease they did not appear to be causally rela

(10) A number of patients were studied in whom jaundice develop the course of anti syphilitic treatment with organic arsenicals, after fusion with blood products, or as a complication of glandular fev malaria The nature of the liver disorder in these conditions is discus

(11) Therapeutic trials with cysteine and methionine suggested that substances may have a beneficial effect on the rate of recovery, but f observations are needed on this point There appeared to be no vir restricting the intake of fat in the diet apart from the distaste of some p for greasy foods

Biochemical Observations

(1) The biochemical tests used to follow the course of the disease we

- (a) serum bilirubin estimation,
- (b) direct quantitative Van den Bergh reaction,
- (c) urine bilirubin estimation by Hunter's test,
- (d) urine bilirubin estimation by iodine ring test,
- (e) urine urobilin and urobilinogen estimation by spectroscopic test,
- (f) bromsulphalein excretion test,
- (g) intravenous hippuric acid test

(2) Results suggested that liver function was deranged in most cases 10 days before the appearance of jaundice and often very shortly afte onset of symptoms Serum bilirubin and urine urobilin plus urobili remained within normal limits until 2 to 3 days before the appearan jaundice

(3) Abnormal results in some of the tests have been used to dia sub icteric cases of the disease when liver damage may occur

(4) The variation of bilirubinuria, serum bilirubin, urinary urobilin urobilinogen during the disease is summarized in a diagram

(5) Results from the hippuric acid synthesis test indicated that maximu liver damage occurred before peak serum bilirubin level was reached

ry occurred in most cases but occasionally liver damage
ıy months
tation of the results of the Van den Bergh reaction is

Haematological Investigations

early sub icteric cases of infective hepatitis from
neutropenia and slight lymphopenia with a relative
is ... ved ard more than the normal number of plasma cells
seen

Service personnel seen soon after jaundice appeared the blood picture
ilar except that there was an absolute monocytosis and fewer cases
ed an abnormal number of plasma cells

A marked increase in large lymphocytes occurred in the early stages
disease with a fall of small lymphocytes Convalescent Service cases
ed a slight lymphocytosis and the proportions of large and small
hocytes returned to normal about 6 weeks after the onset when the
blood picture was approaching normal

Abnormalities in erythrocyte counts and haemoglobin and haematocrit

le salts to the blood might cause an inhibition of the erythrocyte
nentation rate in the acute phase of the disease

Other conditions such as rubella and malaria which may be confused
the blood picture of infective hepatitis are discussed

Serological Investigations

(1) Saline extracts of livers from fatal cases of homologous serum infective
r arsenotherapy hepatitis were used as antigens in complement fixation
ests against sera from acute and convalescent cases of all three diseases
Extracts of normal livers were used as controls

(2) Sera from convalescent cases of arsenotherapy hepatitis during an
hich reacted with liver
those from other types
were obtained

(3) A complement fixation reaction occurred between early specimens of
era from all three types of hepatitis and extracts from normal and abnormal
ivers This was not observed when convalescent sera were used

(4) High titres of agglutinins for sheep s erythrocytes were reported in cases
ho developed homologous serum hepatitis after receiving dried reconstituted
or not No abnormal titres were found in sera
arsenotherapy hepatitis nor in fresh icterogenic

sted a different causation for infective hepatitis
s serum or arsenotherapy hepatitis but there is
evidence for or against cross immunity between the latter two conditions

Transmission Experiments

(1) Attempts to transmit infective hepatitis to normal golden [illegible] Orkney voles, cotton rats, young guinea-pigs, pregnant guinea [illegible] their foetuses, canaries and mice, and pigs on deficient diets were u[illegible]

(2) Blood, faeces and urine collected from cases of in[illegible]vations during inoculated into rats on a low protein diet Tissues fr[illegible]sted, but these used for inoculations into more rats and caused [illegible] Residual signs inc haemorrhages in the lungs, stomach, intestines [illegible] inflammation of the spleen The symptoms failed to [illegible] after [illegible] passage The results could not be confirmed

(3) Human volunteers received nasal, oral or parenteral administ[illegible] of nasopharyngeal washings, serum, urine and faeces collected from c[illegible] infective hepatitis Positive results were obtained from nasal an[illegible] administrations of faecal suspensions or inoculations of serum

(4) Homologous serum hepatitis was transmitted by injection b[illegible] by nasal or oral administration of icterogenic serum

(5) The active icterogenic agent present in cases of arsenotherapy h[illegible] could be transmitted by subcutaneous injection of serum, but not b[illegible] administration of faecal suspensions It was very resistant to heat a[illegible] weaker antiseptics, and the use of inadequately sterilized syringes be a factor in its transmission

(6) Infective hepatitis is believed to be due to a virus called virus A. is evidence of its presence in faeces from cases several days befo[illegible] appearance of jaundice until 4 days afterwards

(7) Virus B causing homologous serum hepatitis has not been in faeces

(8) The derivation of virus B and its possible relation to virus A re[illegible] undecided